Genetic mutations in the treatment of anaplastic thyroid cancer: a systematic review

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Abstract

Background: Anaplastic thyroid cancer (ATC) is a rare, lethal disease associated with a median survival of 6 months despite the best multidisciplinary care. Surgical resection is not curative in ATC patients, being often a palliative procedure. Multidisciplinary care may include surgery, loco-regional radiotherapy, and systemic therapy. Besides conventional chemotherapy, multi kinase-targeted inhibitors are emerging as novel therapeutic tools. The numerous molecular alteration detected in ATC are targets for these inhibitors. The aim of this review is to determine the prevalence of the major genetic alterations occurring in ATC and place the results in the context of the emerging kinase-targeted therapies.

Methods: The study is based on published PubMed studies addressing the prevalence of BRAF, RAS, PTEN, PI3KCA and TP53 mutations and RET rearrangements in ATC.

Results: 21 articles dealing with 652 genetic analyses of the selected genes were used. The overall prevalence determined were the following: RET/PTC, 4%; BRAF, 23%; RAS, 60%; PTEN, 16%; PI3KCA, 24%; TP53, 48%. Genetic alterations are sometimes overlapping.

Conclusions: Mutations of BRAF, PTEN and PI3KCA genes are common in ATC, with RAS and TP53 being the most frequent. Given ATC genetic complexity, effective therapies may benefit from individualized therapeutic regimens in a multidisciplinary approach.

Introduction

Thyroid cancer is the most prevalent endocrine malignancy accounting for 1% of cancers worldwide. More than 95% of thyroid cancer are well differentiated tumors that respond to surgery followed by radioactive iodine (RAI) therapy and thyroid hormone suppression. Although disease recurrence occurs in approximately 30% of cases, nowadays thyroid cancers have a very favorable outcome. The clinical appearance of thyroid cancer is that of a nodules, some time representing a challenging diagnostic dilemma with thyroid or unusual extrathyroidal masses [1,2]. The use of effective diagnostic tools such as ultrasound (US) and fine-needle cytology (FNC) [3-5] has increased the detection of small and well differentiated tumors in their early stages. Moreover, the application of molecular techniques to FNC has dramatically increased its sensitivity [3,4,6-9]. An effective FNC diagnosis avoids useless diagnostic surgery or provides indications for the proper surgical treatment, when needed [10,11]. Poorly differentiated subtypes, including anaplastic thyroid cancer (ATC), are resistant to RAI and conventional chemotherapy. ATC accounts for about 1% of thyroid cancer and is typical of old age. When feasible, surgery must aim at a radical intent; however, surgical resection is not curative in ATC patients, being often a palliative procedure [10,11]. Therefore, an early and accurate diagnosis is mandatory in case of ATC which does not require surgical treatment, and even more in elderly patients, for whom surgery is generally more burdensome, complex and expensive than younger patients [10,11]. Standard chemotherapies have systemic toxicities and limited...
efficacy in the case of ATC as well as of other more common solid tumors [12-14]. Alternative strategies such as immunotherapy are under investigation, but still far from clinical practice [15]. At present, genetic-based targeted therapy is the most promising curative strategy. Hallmarks of all cancers are self-sufficiency in growth signals and evasion of programmed cell death. Tyrosine kinase receptors/ RAS/RAF/MAPK and RAS/PI3K/Akt/mTOR are the major signaling pathways involved in cell proliferation, protein synthesis and cell survival. Thyroid cancer is characterized by several genetic alterations along these two pathways, including rearrangements of the RET (rearranged during transfection; RET/PTC) tyrosine receptor kinase, activating point mutations in the BRAF serine/threonine kinase, in the RAS proto-oncogenes, in the catalytic subunit of the phosphatidyl-inositol 3-Kinase (PI3KCA), or inactivating mutations in the tumor suppressors phosphatase and tensin homolog (PTEN) and TP53 (Table 1). ATC is the product of the accumulation of genetic alterations due to genetic instability and external factors such as food or environmental factors, including ionizing radiations and oxidative stress. Oxidative stress has been implicated in the mechanism of cancer, diabetes, cardiovascular and other diseases [16,17]. Oxidant molecules are generated by stress agents such chemicals, drugs, pollutants, and high-caloric diets [18]. Conversely, there is no hint of a remodeling of the Ca2+ toolkit, that has been observed in other malignancies, including renal cellular carcinoma [19-21], and prostate cancer [22], and has been put forward as alternative target for selective molecular therapies [14]. The last decade has seen advances in the understanding of the molecular basis of thyroid cancer, leading to the application of new pharmacological treatments with inhibitors of kinases [23-25]. These drugs are multi-target agents with inhibitory activity of receptors involved in the angiogenesis or inhibitors of kinases involved in thyroid cancer development. The BRAF inhibitor vemurafenib (PLX4032) improves survival among patients with metastatic melanoma, and suppresses growth of BRAF-mutated human ATC in a mouse model [26]. The beneficial effect of BRAF inhibition in ATC with activating BRAF mutations has been recently reported [27].

### Materials and methods

A meta-analysis was performed by searching the MEDLINE database (National Library of Medicine, Bethesda, MD) using the terms “BRAF”, “RAS”, “PTEN”, “PI3KCA”, “TP53”, “RET/PTC” or ’BRAF,” associated with the terms “anaplastic thyroid cancer” or “undifferentiated thyroid cancer”. Studies were included only when the sample was ≥ 4. Studies were selected on the basis of the detection of molecular alterations by genetic analysis. Studies based only on molecular detection by immunohistochemistry were excluded. Only data about different genes were included from studies by the same authors. Studies on poorly differentiated thyroid cancers and well differentiated thyroid cancers were also excluded.

### Results

The literature search strategy retrieved 104 articles from PubMed. Twenty-one studies met the inclusion criteria and were considered for further analysis. These studies were published between 1993 and 2010, and included 652 cases of ATC. All studies were retrospective, using stored formalin-fixed paraffin-embedded samples or frozen surgical specimens. The method used for determining the presence of single point mutations was direct sequencing of DNA after polymerase-chain reaction (PCR) amplification, PCR and fluorescence melting curve analysis and DNA-mutant allele-specific amplification (DNA-MASA). The methods used to determine RET rearrangements were PCR alone followed by direct sequencing or PCR followed by internal probe binding (Southern blot on PCR products). BRAFV600E was the only BRAF mutation considered by the 7 studies analyzed. The mutation ranged 0%-50% in 21 out of 89 tumors (Table 2). The mean prevalence was 23%. Mutations in the three RAS isoforms ranged 8%-60% in 33 out of 162 ATCs (mean 60%). Not all the three major RET rearrangements were considered in all studies. Tumors were tested for the presence of RET/PTC-1 and -3 in two studies and RET/PTC-1, -2, and -3 in one study. Rearrangements were rare, being detected in 4% of ATCs, in the range 0%-6% in 3 out of 81 tumors. Inactivating mutations of PTEN were detected in 16% of 107 ATCs, while activating mutations of PI3KCA in 23% of 70 ATCs in the range 12%-58% (Table 3). Inactivating mutations of TP53 were identified in 48% of 25 tumors, in the range 10%-86%.

### Table 1 Gene mutations in ATC

| Gene | Mutation | Signaling involvement |
|------|----------|-----------------------|
| RET  | Recombination | MAPK activation |
| BRAF | Single point mutation | MAPK activation |
| H-, N-, K-RAS | Single point mutation | MAPK, PI3K/Akt/mTOR activation |
| PTEN | Single point mutation/deletion | PI3K/Akt/mTOR inactivation |
| PI3KCA | Single point mutation | PI3K/Akt/mTOR activation |
| TP53 | Single point mutation | P53 pathway inactivation |

Other pharmacological compounds inhibit RET and RET/PTC (sorafenib, sunitinib, vandetanib) or the mammalian target of rapamycin (mTOR), a component of the PI3K/Akt signaling pathway (everolimus). Hence, the knowledge of the tumor mutation status is needed for optimizing and tailoring the treatment with kinase inhibitors. The intent of this systematic review is to determine the prevalence of the major genetic alterations occurring in ATC.
Discussion

The prognosis of differentiated thyroidal tumors is generally favorable mainly because there are different and effective tools in the early diagnosis and treatment of these tumors [28]. In fact, the use of US and FNC in the diagnosis of thyroid nodules usually leads to an early and accurate diagnosis of small and differentiated tumors, as well as less frequent thyroidal neoplasms [3,5,6]. In particular, FNC, coupled with immunocytochemistry (ICC), flow cytometry (FC) and molecular techniques [3-6,29-31] has dramatically enhanced the sensitivity and the accuracy of preoperative diagnosis of thyroidal nodules [3,5,29]. The bad prognosis of advanced thyroid carcinoma, prompted researchers to evaluate the efficacy of new pharmaceutical compounds with enzymatic inhibitory properties (Table 4). The prevalence of RET/PTC rearrangements in ATC was much lower than in papillary thyroid cancer reported in most of the studies (4% vs. 36%) [25,32]. Noteworthy, benign thyroid nodules exhibiting RET/PTC rearrangements do not evolve in cancer [33,34]. This data suggest that this oncogene has a minor role in the progression from well-differentiated to undifferentiated thyroid cancer. It also indicate that tyrosine kinase inhibitors such as sorafenib, sunitinib, and vandetanib have little chance to function through the inhibition of this oncogene in ATC. The encouraging results obtained by these drugs in non RAI-responsive differentiated thyroid carcinomas in some clinical trials where the RET rearrangement was not evaluated, were more likely due to the effects on neo-angiogenesis [35]. The high prevalence of BRAFV600E mutation in ATC supports the hypothesis that many ATCs actually represent a progressive malignant degeneration of BRAF-mutated, well-differentiated thyroid carcinomas [36]. This gene is a pivotal component of the MAPK pathway and reduces the activity of p21kip1 in thyroid tumors, stimulating the cell cycle machinery [37]. Vemurafenib (PLX4032), a BRAF selective kinase inhibitor and sorafenib, a multi-target inhibitor, find application in selected BRAF-mutation positive melanomas [38]. Although clinical studies of BRAF inhibitors in advanced non RAI-responsive differentiated thyroid carcinomas have shown encouraging results with frequent early responses, in a relevant fraction of patients this effect was of limited duration, with frequent relapse or no response. In addition, intratumoral heterogeneity with respect to BRAF mutation makes the evaluation of these clinical trials even more

| Table 2 Prevalence of mutations in the MAPK pathway in ATC |
|----------------|-----------------|----------------|------|
| Mutation       | Positive/ total cases | Prevalence (%) | Reference |
| BRAFV600E      | 0/7              | 0              | [51] |
|                | 2/6              | 33             | [52] |
|                | 3/29             | 10             | [53] |
|                | 2/10             | 20             | [54] |
|                | 8/16             | 50             | [55] |
|                | 0/4              | 0              | [56] |
|                | 6/17             | 35             | [57] |
| Overall BRAFV600E | 21/89           | 23             |      |
| RAS            | 4/50             | 8              | [44] |
|                | 2/18             | 11             | [58] |
|                | 1/5              | 20             | [59] |
|                | 4/18             | 23             | [43] |
|                | 15/29            | 55             | [60] |
|                | 4/50             | 8              | [61] |
|                | 3/5              | 60             | [62] |
| Overall RAS mutations | 33/162      | 20             |      |
| RET/PTC        | 0/14             | 0              | [63] |
|                | 3/51             | 6              | [44] |
|                | 0/17             | 0              | [64] |
| Overall RET/PTC | 3/81            | 4              |      |

| Table 3 Prevalence of mutations not in the MAPK pathway in ATC |
|----------------|-----------------|----------------|------|
| Mutation       | Positive/ total cases | Prevalence (%) | Reference |
| PTEN           | 8/48             | 17             | [44] |
|                | 8/50             | 16             | [61] |
|                | 1/9              | 10             | [65] |
| Overall PTEN   | 17/107           | 16             |      |
| PI3KCA         | 6/50             | 12             | [44] |
|                | 4/18             | 22             | [58] |
|                | 29/50            | 58             | [61] |
|                | 16/70            | 23             | [66] |
| Overall PI3KCA | 45/188           | 24             |      |
| TP53           | 1/11             | 10             | [67] |
|                | 5/7              | 71             | [19] |
|                | 6/7              | 86             | [68] |
| Overall TP53   | 12/25            | 48             |      |

| Table 4 Major pharmaceutical compounds in clinical development for the treatment of thyroid cancer |
|------------------------------------------------|-----------------|----------------|--------------|
| Pharmaceutical compound                      | VEGFRs | RET/PTC | BRAF | PDFGR | mTORC1 |
| Axitinib                                    | +      | -      | +   | -    | -      |
| Cabozantinib                                | +/-    | -      | -   | -    | -      |
| Lenvatinib                                  | +      | -      | +   | -    | -      |
| Motesanib                                   | +      | -      | -   | +    | -      |
| Pazopanib                                   | +      | -      | -   | +    | -      |
| Sorafenib                                   | +      | +      | +   | -    | -      |
| Sunitinib                                   | +      | +      | -   | +    | -      |
| Vandetanib                                  | +      | +      | -   | -    | -      |
| Vemurafenib                                 | -      | -      | +   | -    | -      |
| Everolimus                                   | -      | -      | -   | -    | +      |
Conclusions

ATC is characterized by genomic instability that leads to mutations in RET, BRAF, RAS, PTEN, PIK3CA and TP53 genes. The survival of ATC patients has changed little in the past 50 years, despite the introduction of new therapeutic tools. Given the complexity of the genomic alterations of ATC, therapy results may benefit from individualized therapeutic regimens that maximally inhibit major pathways. In the future, these therapies may be successful with a multidisciplinary approach.
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