Anaplastic thyroid carcinoma: Current issues in genomics and therapeutics

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Abstract

Purpose of review Anaplastic thyroid carcinoma is a type of thyroid carcinoma with the most aggressive biological behaviour amongst thyroid cancer. Here, we review the current genomic and the impacts of advances in therapies to improve the management of patients with the cancer.

Recent findings Common mutations being identified in anaplastic thyroid carcinoma are *p53* and *TERT* promoter mutations. Other common mutated genes included *BRAF*, *RAS*, *EIF1AX*, *PIK3CA*, *PTEN*, and *AKT1*, *SWI/SNF*, *ALK* and *CDKN2A*. Changes in expression of different MicroRNAs also involved in the pathogenesis of anaplastic thyroid carcinoma. Curative resection combined with radiotherapy and combination chemotherapies (such as anthracyclines, platins and taxanes) has shown to have effects in the treatment of some patients with anaplastic thyroid carcinoma. Newer molecular targeted therapies in clinical trials target mostly the cell membrane kinase and downstream proteins. These include targeting the EGFR, FGFR, VEGFR, c-kit, PDGFR, RET on the cell membrane as well as VEGF itself and the downstream targets such as BRAF, MEK and m-TOR. Immunotherapy is also being tested in the cancer.

Summary Updated knowledge of genomic as well as clinical trials on novel therapies are needed to increase the improve the management of the patients with this aggressive cancer.

Keywords: anaplastic thyroid carcinoma; treatment; genomics; BRAF; TERT

1.Introduction

Anaplastic thyroid carcinoma is one of the most lethal human carcinomas [1]. The carcinoma responds very poorly to conventional treatments. The recent development of next-generation sequencing revealed more information about genetic profiles on anaplastic thyroid carcinoma. The genomic of the carcinoma is complex and difficult to be targeted in the current clinical practices. Nevertheless, these genomic data are useful for the future advances of treatment for anaplastic thyroid carcinoma. In this article, we review the new development in genomics and the application of these data to complement the advance of treatment in patients with anaplastic thyroid carcinoma.

2.Genomic changes in anaplastic thyroid carcinoma

Several next-generation sequencing studies have been performed recently, including whole-genome sequencing study [2*], whole-exome sequencing studies [3, 4], and targeted sequencing studies [5-14] to study the explore the genetic changes in anaplastic thyroid carcinoma. The mainstream of the molecular mechanism of anaplastic thyroid carcinoma was summarised in Figure 1. As anaplastic thyroid carcinoma is one of the most aggressive human cancer, it follows that the genomics changes of anaplastic thyroid carcinoma are much complex and different from the more

indolent well-differentiated thyroid carcinoma, e.g. papillary thyroid carcinoma. Table 1 lists the frequencies of the common mutations found in the anaplastic thyroid carcinoma.

The most frequently identified mutations in anaplastic thyroid carcinoma are *p53* and *telomerase reverse transcriptase (TERT)* promoter mutations. *p53* is the best-known tumour suppressor gene. *p53* protein regulates intracellular pathways in cell survival, DNA-repair, apoptosis and senescence. Mutations in *p53* induce tumour progression with loss of function of *p53* in various malignant tumours [15-18]. Overexpression of *p53* protein was seen in anaplastic thyroid carcinoma with high rate [1]. *p53* mutations were detected in large numbers of patients with anaplastic thyroid carcinoma with next-generation sequences (range = 27% to 78%, Table 1).

TERT promoter mutations were also frequently detected in patients with anaplastic thyroid carcinoma (range = 32% to 73%, Table 1). The mutations provide *TERT* reactivation, which induces unlimited cellular proliferation essential for transformation in cancer cells [19]. In papillary thyroid carcinoma, *TERT* promoter mutations are associated with poor patient survival rate [20]. Shi and co-workers on working on 106 anaplastic thyroid carcinomas showed the *TERT* promoter mutations

were associated with that of *BRAF* mutations as well as distant metastasis and patients' advanced age in patients [21].

In anaplastic thyroid carcinoma, there are two major cascades in tumorigenesis, the RAS-BRAF-MEK pathway and PI3K-AKT-mTOR pathway. In RAS-BRAF-MEK pathway, *RAS* mutations and *BRAF* mutations are common in patients with anaplastic thyroid carcinoma. *RAS* family includes *HRAS*, *KRAS*, and *NRAS*. In thyroid carcinomas, these mutations were often detected particularly in follicular thyroid carcinoma as well as follicular variant of papillary thyroid carcinoma [22, 23]. Next-generation sequencing study revealed *RAS* mutations were detected in patients with anaplastic thyroid carcinoma (range = 0% to 43%, Table 1). This could indicate some anaplastic thyroid carcinoma may be developed from follicular thyroid carcinoma.

Landa and co-workers revealed *eukaryotic translation initiation factor 1A X-linked (EIF1AX)* mutations were highly associated with *RAS* mutations [10]. *EIF1AX* mutations were detected in up to one-third of anaplastic thyroid carcinoma (range = 0% to 33%, Table 1) [2*, 3, 4, 9, 10]. The function of *EIF1AX* on malignancies is not fully understood.

BRAF V600E mutations are common in anaplastic thyroid carcinoma (range = 7% to 91%, Table 1). Khan and co-workers showed 94 % of *BRAF* mutations were

BRAF V600E mutations in patients with anaplastic thyroid carcinoma [7]. *BRAF* regulates cell division and survive, and *BRAF V600E* mutations play an important role for development and progression of various malignant tumours including anaplastic thyroid carcinoma [24-29].

BRAF mutation is the most common mutation in conventional papillary thyroid carcinoma [30]. Multiple proliferation-survival signalling pathways are simultaneously active in *BRAF* mutated papillary thyroid carcinoma or anaplastic thyroid carcinoma [31]. The mutation is associated with increased mortality and recurrence of papillary thyroid carcinoma [32-37]. Yoo and co-workers showed that metastatic papillary thyroid carcinoma had *BRAF* mutations with similar prevalence to anaplastic thyroid carcinoma, whereas widely invasive follicular thyroid carcinoma had no *BRAF* mutations [2*]. This report revealed most of *BRAF V600E* mutations were detected in papillary thyroid carcinoma, which indicated the genomic relationship between papillary thyroid carcinoma and anaplastic thyroid carcinoma.

In the PI3K-AKT-mTOR pathway, mutations of *PIK3CA*, *PTEN*, and *AKT1* were detected in patients of anaplastic thyroid carcinoma. *PIK3CA* encodes p110-alpha protein which is the catalytic subunit of a phosphatidylinositol 3-kinase (PI3K) [38]. *PIK3CA* mutations were associated with tumour prognosis in various cancers

with the increase of cell signalling, cell growth, and invasion [39, 40]. In addition, studies indicated associations between *PIK3CA*, *BRAF* and *RAS* mutations [41, 42]. In anaplastic thyroid carcinoma, *PIK3CA* mutations were detected (range = 6 to 44%, Table 1). The mutation is more frequently detected than in differentiated thyroid carcinomas (papillary and follicular thyroid carcinomas) and poorly differentiated thyroid carcinomas [5, 10].

PTEN (*Phosphatase and tensin homolog*) encoded PTEN enzyme, which acts as a tumour suppressor by regulating cell division and preventing abnormal cell growth and division [43]. *PTEN* mutations were detected in patients with anaplastic thyroid carcinoma (range = 0% to 18%, Table 1). [2*, 3-5, 7-11, 13, 14]. The mutations were detected more frequently in anaplastic thyroid carcinoma than papillary thyroid carcinoma or poorly differentiated thyroid carcinoma. The frequency of *PTEN* mutations in follicular thyroid carcinoma and Hurthle cell carcinoma was like that of anaplastic thyroid carcinoma [5, 10]. Meanwhile, two studies of next-generation sequence on anaplastic thyroid carcinoma revealed there were no patients with *PTEN* mutations [3, 11]. This controversy indicates the genomic diversity of anaplastic thyroid carcinoma.

AKT1 (*Protein kinase B, PKB*) encodes AKT1 kinase, which regulates cell growth, proliferation, and differentiation [44]. *AKT1* mutations were detected in patients with anaplastic thyroid carcinoma but with lower rate than mutations of *PI3CA* or *PTEN* (range, 0% to 14%, Table 1) [2*, 3-5, 7-9, 13, 14].

In addition to mutations of two pathways, the other mutations were detected. *SWI/SNF* gene mutations were sometimes detected in patients of anaplastic thyroid carcinoma (range = 0% to 27%, Table 1) [2*, 3-5, 7, 10, 14]. SW1/SNF complex acts as a tumour suppressor and loss of SW1/SNF function causes abnormal gene silencing and induces tumour progression [45, 46]. The role of *SWI/SNF* mutations in anaplastic thyroid carcinoma remains unclear.

Some patients with anaplastic thyroid carcinoma had *CDKN2A* (*cyclin-dependent kinase inhibitor 2A*) mutations (range = 0% to 32%, Table 1) [2*, 3-5, 7, 11, 12, 14]. *CDKN2A* encodes p16 protein, a well-known tumour suppressor. Loss of p16 protein caused by *CDKN2A* mutations are associated with tumour progression and poor prognosis in patients with anaplastic thyroid carcinoma (as well as poorly differentiated thyroid carcinoma and metastatic papillary thyroid carcinoma) [2*]. This indicated mutations of *CDKN2A* could play an important role in anaplastic thyroid carcinoma.

ALK (*anaplastic lymphoma kinase*) mutations and fusions were detected in a small portion of anaplastic thyroid carcinomas (range = 0% to 20%, Table 1) [2*, 3-10, 12-14]. *ALK* rearrangements caused by mutations and fusions have been shown to activate both RAS-BRAF-MEK pathway and PI3K-AKT-mTOR pathway in cancers, such as non-small cell lung carcinoma [47-49]. The role of *ALK* rearrangements in anaplastic thyroid carcinoma remains unclear.

RET (*receptor tyrosine kinase*) rearrangements caused by fusions were detected in anaplastic thyroid carcinoma [50]. However, recent genomic studies were not able to detect this rearrangement. *RET* fusions were sometimes detected in patients with papillary thyroid carcinoma [51]. Thus, patients with anaplastic thyroid carcinoma having a history of well-differentiated thyroid carcinoma could have *RET* rearrangements.

MicroRNAs are single-stranded non-coding RNAs and regulate their target genes expression, which associates with tumour progression of various tumours [52-60]. Downregulated or upregulated microRNAs could affect tumour progression. Several microRNAs having the potency of tumour prognosis in anaplastic thyroid carcinoma were reported. MicroRNA-146a/b, microRNA-221/222, and cluster microRNA-17-92 were demonstrated to be upregulated in anaplastic thyroid carcinoma. On the other

hand, microRNA-200 family, microRNA-30 family, let-7 family were noted to be downregulated in anaplastic thyroid carcinoma. These microRNA alterations were reported to associate cell proliferation, cell growth, and cell differentiation by affecting various molecules, such as PTEN (microRNA-146a/b, cluster microRNA-17-92), p27^{kip1}(microRNA-221/222), p53 (microRNA-146a/b, microRNA-200 family), Beclin1 (autophagy-promoting protein; microRNA-30 family) and RAS (let-7 family) [61-68].

Studies have revealed the mechanisms of microRNAs in the progression of anaplastic thyroid carcinoma. Upregulated microRNA-195 suppressed progression of anaplastic thyroid carcinoma by affecting VEGF and p53 [69]. Upregulated microRNA-34b, microRNA-205, and microRNA-126 reduced tumour growth by suppressing angiogenesis by reduction of VEGF and apoptosis in anaplastic thyroid carcinoma [70-73]. Hence, studies of the roles of microRNA in anaplastic thyroid carcinoma could provide potential useful information for target therapeutic approaches on anaplastic thyroid carcinoma.

3. Treatment of anaplastic thyroid carcinoma

3.1. Surgery

Patients with surgical resection had a significantly longer overall survival rate than those without surgical resection [74]. Curative resection is of benefit to the patients with anaplastic thyroid carcinomas [75] but is limited to patients with localised disease (Stage IVA and a small part of patients with Stage IVB disease). Patients who had debulking surgery had a marginally longer median overall survival rate than those who had not (3.0 months vs 2.1 months) [76]. After curative resection, use of adjuvant chemotherapy and radiotherapy was recommended by the American Thyroid Association for the treatment of anaplastic thyroid carcinoma [77]. Patients with surgical resection of anaplastic thyroid carcinoma had a more effective response for concomitant treatments of radiation and chemotherapy and had longer median overall survival rate than patients without surgical resection (43.0 months vs 3.3 months) [78]. Hence, surgery, particularly curative resection, should be performed whenever possible in this cancer.

3.2 Radiotherapy and chemotherapy

Patients having anaplastic thyroid carcinoma and treated with radiotherapy had a significantly longer overall survival rate than those not treated by radiotherapy [74].

Radiotherapy is seldom performed as the sole therapy for anaplastic carcinoma. By the same token, it is also uncommon to perform chemotherapy alone. Nevertheless, a few patients with anaplastic thyroid carcinoma who have received chemotherapy without radiotherapy achieved complete response [79, 80]. Overall, the combination of chemotherapy and radiotherapy as the adjuvant treatment after surgery or as the initiative treatment of the inoperable condition should be recommended for patients with anaplastic thyroid carcinoma.

Regarding chemotherapy, anthracyclines (doxorubicin), platins (cisplatin), and taxanes (docetaxel and paclitaxel) have been shown to be effective in the treatment of patients with anaplastic thyroid carcinoma.

Doxorubicin induces cytotoxicity in cancer cells and prevents tumour prognosis by acting as the suppressor of DNA polymerase, DNA topoisomerase II, and DNA methyltransferase [81] whereas cisplatin induces apoptosis in cancer cells by interfering with DNA repair mechanisms which lead to DNA damage [82]. Shimaoka and co-workers reported complete response and partial response in 0 % and 5% of patients with anaplastic thyroid carcinoma who were treated by doxorubicin alone, whereas complete response and partial response were seen in 17 % and 17 % of patients who were treated by the combination of doxorubicin and cisplatin. The median overall

survival of all patients was 2.7 months [83]. There were no reports about the efficacy of cisplatin alone in patients with anaplastic thyroid carcinoma.

Taxanes (docetaxel and paclitaxel) were reported to arrest cell division by inhibiting microtubular depolymerisation [84]. In addition, both docetaxel and paclitaxel increased the cell fraction in the G2/M phase of the cell cycle, considered to be the most radiosensitive phase, leading to radiation-sensitising effects [85, 86]. Complete response and partial response were seen in 0 to 8 % and 21 to 47% of patients who were treated by paclitaxel with or without radiotherapy, respectively [79, 80, 87]. In addition, Troch and co-workers reported that 83% of patients with anaplastic thyroid carcinoma who received docetaxel therapy and radiotherapy were alive after a median follow-up time of 21.5 months. Furthermore, 67% of the patients achieved complete response and the other 33% of patients achieved partial response that was confirmed by imaging analysis and clinical follow up [88]. Accordingly, chemotherapy with taxanes plus radiotherapy may be an effective treatment for patients with anaplastic thyroid carcinoma.

3.3 Targeted therapy

With the understanding of genomic profiles of anaplastic thyroid carcinoma, molecular targeted therapy could be effective. Various agents have been investigated (Figure 1). The targets for therapy are listed in Table 2. These include targeting the EGFR (epidermal growth factor receptor), FGFR (fibroblast growth factor receptor), VEGFR (vascular endothelial growth factor receptor), c-kit (cluster of differentiation 117), PDGFR (platelet-derived growth factor receptor), RET (rearranged during transfection) on the cell membrane as well as VEGF (vascular endothelial growth factor) itself and the downstream targets such as BRAF, MEK and m-TOR.

Gefitinib is an epidermal growth factor receptor (EGFR) inhibitor, which has an inhibitory effect both on the autophosphorylation and downstream signalling, competing with the adenosine triphosphate (ATP) for the catalytic domain of EGFR, and leads to apoptosis in cells with mutated and overactive EGFR [89]. In vivo and in vitro study revealed that high EGFR expression was found in anaplastic thyroid carcinoma cells (ACT-1 cell line). Gefitinib inhibited cell proliferation of these anaplastic thyroid carcinoma cells as well as on the growth of anaplastic thyroid carcinoma transplanted in mice [90]. Gefitinib was first tested as the agent of targeted therapy, but none of the patients achieved complete/partial response [91].

Imatinib, is an inhibitor of bcr-abl fusion protein, platelet-derived growth factor receptor (PDGFR), and c-kit. This agent has the competitive inhibitory effect at ATP binding site of tyrosine kinase enzymes, which leads to inhibition of tyrosine phosphorylation of them [92], and this effect is specific for bcr-abl, PDGFR, and c-kit in various tyrosine kinase enzymes [93]. Podtcheko and co-workers showed imatinib inhibited cell growth of anaplastic cancer cells with mutated or undetectable p53 in vitro by inhibiting abl kinase [94]. Meanwhile, Dziba and Ain reported imatinib had negligible antineoplastic activity, and no constitutive kinase activity was detected in cells of anaplastic thyroid carcinoma [95]. Aberrant expression of PDGFR was also reported in anaplastic thyroid carcinoma cells [96]. Hence, imatinib was tested for patients with anaplastic thyroid carcinoma having PDGFR overexpression, and 25% of patients achieved partial response while 0% of patients achieved complete response and 45% of patients had 6-month overall survival [97].

Pazopanib is an inhibitor of vascular endothelial growth factor receptor (VEGFR), PDGFR, and c-kit. Pazopanib suppresses the tumour angiogenesis and tumour cell proliferation by ATP-competitive inhibition and ligand-induced autophosphorylation of VEGFR, PDGFR, and c-kit [98, 99]. Bible and co-workers using xenograft studies of anaplastic thyroid carcinoma indicated that pazopanib

reduced tumour volume. However, in the clinical trial, 100% of patients treated had progressive disease and median overall survival was 3.7 months [100].

Axitinib is a selective inhibitor of VEGFR with ATP-competitive inhibition like pazopanib, and its potency against VEGFR was reported to be higher than that of pazopanib [101]. Axitinib was tested for advanced or metastatic thyroid carcinoma including two patients with anaplastic thyroid carcinoma. One patient with anaplastic thyroid carcinoma achieved partial response though another one showed progressive disease [102].

Sunitinib is an inhibitor of VEGFR, PDGFR and c-kit. Inhibition of VEGFR and PDGFR by sunitinib leads to the reduction of vascularisation of cancer and cancer cell apoptosis [103]. In addition, inhibition of c-kit by this agent is used to treat gastrointestinal stromal cell tumours [104]. In anaplastic thyroid carcinoma, Di Desidero and co-workers showed sunitinib had antiproliferative and proapoptotic activity in anaplastic thyroid carcinoma cells and xenograft model of anaplastic thyroid carcinoma by the inhibition of AKT and ERK1/2 phosphorylation and through the downregulation of cyclin-D1 [105]. In the clinical trial on patients with anaplastic thyroid carcinoma, complete response or partial response were 0% and median overall survival was 5.7 months. [106].

Sorafenib is an inhibitor of BRAF, CRAF, VEGFR, PDGFR, RET, and c-kit.

Sorafenib has dual anti-tumour activity; inhibition of RAS-RAF-MEK signalling pathway and receptor tyrosine kinases, such as VEGFR [107]. Kim and co-workers showed sorafenib inhibited angiogenesis and tumour growth of anaplastic thyroid carcinoma in vivo and in vitro study [108]. However, the clinical trials did not demonstrate a clear efficacy. None of the patients with anaplastic thyroid carcinoma achieved complete response, 0 to 10% of patients achieved partial response and median overall survival of patients was 1.9 to 2.8 months [109, 110]. Kim and co-workers demonstrated that high dose of sorafenib was required to show enough inhibition of cell growth of anaplastic thyroid carcinoma [108]. On the other hand, in vitro study revealed low dose of sorafenib could reduce cell viability and MEK phosphorylation in anaplastic thyroid carcinoma cells with *BRAF V600E* mutations [111]. This might indicate sorafenib could have efficacy for the treatment of anaplastic thyroid carcinoma with *BRAF V600E* mutation.

Lenvatinib is an inhibitor of VEGFR, PDGFR, RET, c-kit, and fibroblast growth factor receptor (FGFR). This agent has been reported to be effective in treatment of differentiated thyroid carcinoma and was approved to for treatment of progressive radioiodine-refractory differentiated thyroid carcinoma by USA Food and Drug

Administration (FDA) and European Medicines Agency (EMA) [112]. Ferrari and co-workers showed lenvatinib reduced cell proliferation, increased the percentage of apoptotic cells, inhibited migration and invasion in anaplastic thyroid carcinoma cells with inhibition of EGFR, AKT and ERK1/2 phosphorylation and downregulated cyclin D1 as well as reduced tumour growth, VEGF expression, and microvessel density in xenograft model of anaplastic thyroid carcinoma [113]. In the clinical trial of patients with anaplastic thyroid carcinoma, the median overall survival of patients with lenvatinib treatment was reported to be 10.4 months, while 0% of patients achieved complete response and 24% of patients achieved partial response [114].

Vemurafenib is an inhibitor of BRAF, which was firstly approved by FDA for the treatment of late-stage melanoma because many patients of advanced melanoma had *BRAF V600E* mutations [115, 116]. In patients with anaplastic thyroid carcinoma, the prevalence of *BRAF V600E* mutations was also high. Zhang and co-workers showed vemurafenib reduced tumour growth in xenograft model of metastatic anaplastic thyroid carcinoma [117]. Complete response rate was 14% and partial response rate was 14% when vemurafenib was tested for patients with anaplastic thyroid carcinoma having *BRAF V600E* mutation [118].

The combination therapy of dabrafenib (an inhibitor of BRAF like vemurafenib) and trametinib (an inhibitor of MEK) was approved by FDA for treatment in patients with anaplastic thyroid carcinoma having *BRAF V600E* mutations. From in vitro study, the combination of dabrafenib and trametinib demonstrated cytostatic effect in anaplastic thyroid carcinoma cells [119]. In clinical trial, Subbiah and co-workers investigated the combined treatment of dabrafenib and trametinib in patients with anaplastic carcinoma who had *BRAF V600E* mutations, and complete response and partial response were seen in 7 % and 67%, and one-year survival rate was reported to be 80% [120*]. Hence, the presence of *BRAF V600E* mutations should be investigated in anaplastic thyroid carcinoma because this combination therapy could be effective to patients of anaplastic thyroid carcinoma with *BRAF V600E* mutations. Further investigation with large cohort should be performed to confirm the efficacy of this therapy.

Everolimus is an inhibitor of mTOR_(mammalian target of rapamycin). mTOR exists in two physically and functionally distinct signalling complexes, mTORC1 and mTORC2 [121]. mTORC1 and mTORC2 each have their own set of downstream targets that modulate cellular growth, angiogenesis and survival in normal cells and cancer cells. Elevated mTOR signalling has been detected in many human

cancers and clinical trials are underway for mTOR inhibitors in cancers [121].

Everolimus is highly selective for the mTORC1 complex and leads to an activation of AKT by inhibition by the mTORC1, which inactivates AKT. Therefore, everolimus could inhibit cell growth and cell proliferation [122]. In anaplastic thyroid carcinoma cells, everolimus reduced cell proliferation [123]. Everolimus has been tested in some clinical studies. None of the patients with anaplastic thyroid carcinoma achieved complete response and 0 to 40% of patients achieved partial response. Median overall survival of patients with everolimus was 4.6-7.4 months [11, 124, 125].

Despite lack of clinical studies, ALK inhibitors (crizotinib, ceritinib, and brigatinib) were reported to be effective for the patient with anaplastic thyroid carcinoma having *ALK* fusion [126, 127]. Selpercatinib, an inhibitor of RET, was also reported to be effective for the patients with *RET*-fusion positive anaplastic thyroid carcinoma co-existing with papillary thyroid carcinoma [128]. These agents for *ALK* or *RET* rearrangements could be useful for some patients with anaplastic thyroid carcinoma.

Some targeted therapies were reported to be effective for anaplastic thyroid carcinoma model but with no evidence from clinical trials. Bevacizumab is an inhibitor of vascular endothelial growth factor (VEGF). This agent has been widely

used for various malignant tumours [129-131]. Garg and co-workers showed bevacizumab reduced tumour growth in xenograft model of anaplastic thyroid carcinoma [132]. Cediranib, an inhibitor of VEGFR, was also reported to reduce tumour growth in xenograft model of anaplastic thyroid carcinoma [133]. These agents have not been tested clinically. Considering the targets of two agents are VEGF and VEGFR, the upper stream of both RAS-BRAF-MEK pathway and PI3K-AKT-mTOR pathway, future clinical trials are warranted.

3.4 Other molecular therapies

Fosbretabulin (combretastatin A-4 phosphate) is a prodrug of the active molecule of combretastatin. It is the microtubule depolymerising agent which affects tumour vascular microenvironments, prevents the tumoral blood supply, and causes necrosis. In clinical trial, the rate of complete response and that of partial response were 0%, and median overall survival of patients with anaplastic thyroid carcinoma was 4.7 months [134].

Efatutazone is PPAR-gamma agonist. PPAR-gamma is nuclear hormone receptors and activation of PPAR-gamma induces antineoplastic effects [135]. From in vitro study on human anaplastic thyroid carcinoma cells, activated PPAR-gamma inhibit proliferation and invasion [136]. Efaturazone was also tested and complete

response rate was 0% and partial response rate was 7% in clinical study. The median overall survival of patients was 98 days (administration of 0.15mg efatutazone orally twice daily) and 138 days (administration of 0.3mg efatutazone orally twice daily) with administration of paclitaxel. [137].

3.5 Immunotherapy

Immune-checkpoint inhibitors are the new potential agents for anaplastic thyroid carcinoma as well as many malignant tumours [138, 139]. In the cancer disease state, the interaction of programmed death ligand (PD-L1) on the tumour cells with programmed cell death protein 1 (PD-1) on T-cell reduces T-cell function to weaken the immune system against the tumour cells. Inhibitors of PD-L1 and PD-1 are effective agents in patients with metastatic cancer [140]. Expression of PD-L1 is used to predict the response of cancer to immunotherapy [141]. In anaplastic thyroid carcinoma, PD-L1 was reported to be the high expression. The prevalence rate of high expression in anaplastic thyroid carcinoma was higher than differentiated thyroid carcinoma [142]. Cantara and co-workers showed by immunohistochemical examination on two antibodies of PD-L1 that PD-L1 was positive in 65% and 90% respectively in anaplastic thyroid carcinoma. In addition, in vivo study showed that

PD-L1 antibody suppressed tumour growth if anaplastic thyroid carcinoma showed expression of PD-L1 [143].

Iyer and co-workers showed the efficacy of pembrolizumab, an anti-PD-1 agent, with lenvatinib, trametinib, or combination of dabrafenib and trametinib for patients with anaplastic thyroid carcinoma. While none of the patients achieved complete response, 42% of patients achieved partial response and median overall was 6.93 months. In this pilot study, the degree of PD-L1 expression was not clearly related to efficacy as well as the concomitant drug with pembrolizumab [144].

Recently, Wang and co-workers investigated 6 patients with anaplastic thyroid carcinoma having *BRAF V600E* mutations who have underwent complete surgical resection and received the combination of dabrafenib and trametinib with or without pembrolizumab [145*]. In the study, local recurrence was not detected in all patients. Patients with pembrolizumab (4 patients) achieved complete remission within the observation period (7.8 to 26.0 months) while patients without pembrolizumab (2 patients) died due to distant metastases. This study indicated the immunotherapy with the combination of dabrafenib and trametinib, which was approved by FDA for treatment in patients with anaplastic thyroid carcinoma having *BRAF V600E* mutations,

could be more effective. Hence, future clinical studies in a larger cohort should be required.

3.6. Complete remission by multimodal treatments in previous reports in detail.

While various treatments have been tested and established, it has been difficult to achieve complete remission of anaplastic thyroid carcinoma. In the recent literature, 9 cases with complete remission within the observation period (at least over 9 months) of anaplastic thyroid carcinoma assessed by pathological examination and/or FDG-PET were reported in detail (Table 3) [146-153]. All the patients with complete remission were of stage IVB and underwent combined treatments of chemotherapy and radiotherapy. Six cases were treated with taxanes (docetaxel or paclitaxel). The cases with complete remission indicated the impotence of performing radiotherapy with chemotherapy for patients without distant metastasis, particularly with taxanes considering the effective combination effect with radiotherapy.

Considering anaplastic thyroid carcinoma is rare, further investigations of the very cases with complete remissions might be important. In addition, from the view of the development of targeted therapy and immunotherapy, future reports of patients with complete remission in detail are expected.

4. Conclusions

Despite the development and diversity of the treatment of anaplastic thyroid carcinoma, optimal treatment for anaplastic thyroid carcinoma has not yet been confirmed. It appears that curative resection of the anaplastic thyroid carcinoma with combination therapies offer the chance of controlling recurrence and the better chance of better patients' survival. The combination therapies may include radiotherapy, chemotherapy (taxanes) and the use of targeted therapies based on the knowledges of complex genomic noted on studies on anaplastic thyroid carcinoma. These targeted therapies such as dabrafenib (an inhibitor of BRAF) and trametinib (an inhibitor of MEK) as well as immunotherapy are of potential in clinical use in patients with anaplastic thyroid carcinoma. Clinical trials on a larger number of cases with the combination of novel therapies are needed to increase the improve the management of the patients with this aggressive cancer.

Figure Legends

Figure 1. Molecular pathogenesis and targeted therapy of anaplastic thyroid carcinoma.

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This study performed whole-genome sequencing in patients with anaplastic thyroid carcinoma.

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Table 1. Recent investigations of mutations in anaplastic thyroid carcinoma using next generation sequencing.

References	Sequencing type	Patient number	Gene mutation										
			<i>p53</i>	<i>TERT</i>	<i>RAS</i>	<i>BRAF</i>	<i>EIF1AX</i>	<i>PI3KCA</i>	<i>PTEN</i>	<i>AKT1</i>	<i>SWI/SNF</i>	<i>CDKN2A</i>	<i>ALK</i>
Pozdeyev <i>et al.</i> 2018	target sequencing	196	65%	65%	27%	41%	NA	14%	11%	2%	18%	22%	1%
Latteyer <i>et al.</i> 2016	target sequencing	30	60%	NA	23%	7%	NA	NA	NA	NA	NA	NA	20%
Khan <i>et al.</i> 2018	target sequencing	90	66%	32%	26%	34%	NA	12%	13%	0%	6%	32%	2%
Jeon <i>et al.</i> 2016	target sequencing	11	73%	NA	9%	91%	NA	18%	9%	0%	NA	NA	0%
Duan <i>et al.</i> 2019	target sequencing	25	60%	56%	24%	56%	8%	44%	16%	4%	NA	NA	0%
Landa <i>et al.</i> 2016	target sequencing	33	73%	73%	24%	45%	9%	18%	15%	NA	36%	NA	0%
Hanna <i>et al.</i> 2018	target sequencing	38	34%	NA	11%	24%	NA	8%	0%	NA	NA	0%	NA
Tiedje <i>et al.</i> 2017	target sequencing	118	55%	73%	20%	11%	NA	12%	NA	NA	NA	17%	0%
Decken-Draissey <i>et al.</i> 2018	target sequencing	9	78%	NA	0%	67%	NA	33%	11%	0%	NA	NA	0%
Bonhomme <i>et al.</i> 2017	target sequencing [#]	144	54%	54%	43%	14%	NA	6%	9%	1%	1%	3%	1%
Kunstman <i>et al.</i> 2015	whole exome sequencing	22	27%	NA	27%	27%	14%	8%	0%	0%	9%	0%	0%
Ravi <i>et al.</i> 2019	whole exome sequencing	14	55%	36%	18%	18%	0%	18%	18%	14%	27%	7%	0%
Yoo <i>et al.</i> 2019	whole genome sequencing*	27	48%	56%	44%	41%	33%	11%	7%	7%	0%	22%	0%

NA: not assessed.

[#]: Target sequencing (next generation sequencing) was performed in 94 patients for assessment except TERT. Sanger sequencing for TERT was performed in 98 patients.

*: Whole genome sequencing was performed in 13 patients and target sequencing was performed in all patients.

Table 2. Overview of studies of targeted treatments for anaplastic thyroid carcinomas.

Agent	Target	References	Number of patients	CR (%)	PR (%)	SD (%)	PD (%)	Overall survival (month)
Gefitinib	EGFR	Pennell <i>et al.</i> 2008	5	0	0	20	80	NA
Imatinib	bcr-abl, PDGFR, c-kit	Ha <i>et al.</i> 2010	8	0	25	50	25	NA
Pazopanib	VEGFR, PDGFR, c-kit	Bible <i>et al.</i> 2012	15	0	0	0	100	3.7
Axitinib	VEGFR	Cohen <i>et al.</i> 2014	2	0	50	0	50	NA
Sunitinib	VEGFR, PDGFR, c-kit	Ravaud <i>et al.</i> 2017 ^Σ	4	0	0	25	NA	5.7
Sorafenib	BRAF, CRAF, VEGFR, PDGFR, RET, c-kit	Savvides <i>et al.</i> 2013	20	0	10	25	65	1.9
		Ito <i>et al.</i> 2017	10	0	0	40	60	2.8
Lenvatinib	VEGFR, PDGFR, RET, c-kit, FGFR	Takahashi <i>et al.</i> 2019	17	0	24	71	6	10.6
Vemurafenib	BRAF	Hyman <i>et al.</i> 2015 [§]	7	14	14	0	57	NA
Dabrafrenib	BRAF	Subbiah <i>et al.</i> 2017 [#]	15	7	67	13	13	NA
Trametinib	MEK		7	0	0	0	100	4.7
Evelolimus	mTOR	Hanna <i>et al.</i> 2018 [¶]	7	0	14	29	43	4.6
		Harris <i>et al.</i> 2019	5	0	40	40	20	7.4

NA: not assessed. CR: complete response, PR: partial response, SD: stable disease, PD: progressive disease, *: 6 patients underwent radiation therapy together. ^Σ: 2 patients did not undergo a subsequent radiological assessment. [§]: 1 patient could not be assessed. All patients had *BRAF V600E* mutations. [#]: All patients had *BRAF V600E* mutations. The rate of 1-year survival was 80%. [¶]: 1 patient could not be assessed.

Table 3. Summary of reported cases with complete remission of anaplastic thyroid carcinoma by multimodal treatments.

References	Chemotherapy	Radiotherapy	Age (years)	Gender	tumour size (cm)	Stage	Confirmation of complete remission	Period of remission
Abe <i>et al.</i> 2015 *	Docetaxel	+	75	Female	7.8	IVB	Pathological (whole body: autopsy)	13 months
Shinohara <i>et al.</i> 2009	Cisplatin, doxorubicin, etoposide, peplomycin	+	53	Female	3.0	IVB	Pathological (neck: surgery) gallium scintigraphy	> 24 months
Noguchi <i>et al.</i> 2009	Cisplatin, doxorubicin, valproic acid	+	51	Male	4.0	IVB	Pathological (neck: surgery) FDG-PET	> 24 months
Zanirato Rambaldi <i>et al.</i> 2016	Carboplatin, paclitaxel	+	68	Female	6.0	IVB	Pathological (neck: surgery) FDG-PET	> 24 months
Zanirato Rambaldi <i>et al.</i> 2016	Carboplatin, paclitaxel	+	33	Male	5.0	IVB	Pathological (neck: surgery) FDG-PET	> 24 months
Pichardo-Lowden <i>et al.</i> 2009	Doxorubicin	+	26	Female	5.0	IVB	FDG-PET	> 24 months
Xing <i>et al.</i> 2011	Doxorubicin, docetaxel	+	56	Male	9.5	IVB	FDG-PET	> 9 months
Koussis <i>et al.</i> 2015	Carboplatin, docetaxel	+	91	Female	1.4	IVB	FDG-PET	> 18 months
Kurukahvecioglu <i>et al.</i> 2007	Docetaxel	+	35	Female	2.1	IVB	FDG-PET	> 36 months

FDG-PET: fluorodeoxyglucose-positron emission tomography, *: the patient died because of suffocation from sputum 13 months after with no worsening of the disease.