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Anaplastic Thyroid Cancer Induced by BRAF V600 and Ras-Raf-MEK-ERK: Promising a New Treatment?

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Abstract Anaplastic thyroid cancer (ATC) is a lethal malignant tumor with rare prevalence. Treatment methods have generally been restricted, but novel inhibitors via clinical trials were explored. In this review, we aim to discuss **ATC** induced by BRAF V600 and Ras-Raf-MEK-ERK mutations. We also emphasized the therapeutic modalities of **ATC** with the help of related inhibitors.

Keywords Anaplastic thyroid cancer, BRAF V600, Ras-Raf-MEK-ERK

1. Introduction

Thyroid cancers are classifyed based on the degree of differentiation including: 1- differentiated thyroid cancer (DTC) with two types comprising papillary thyroid carcinoma (PTC) and follicular thyroid carcinoma (FTC), 2- poorly-differentiated thyroid carcinoma (PDTC) and 3- anaplastic thyroid cancer (ATC) (Li et al. 2019). Anaplastic thyroid cancer (ATC) is a rare tumour containing more than 50% fatality in the thyroid cancers (Corrigan et al. 2019). ATC is one of the most malignant type of thyroid cancers leading to considerable morbidity and mortality. The most important causes of this disease are male gender, age, bilateral tumors, presence of local invasion and distant metastasis (Ragazzi et al. 2014). Incidence is estimated to be 1–2 per million per year (Tiedje et al. 2018). The majority of ATC tumors are inoperable and fatal even during diagnosis (Reddi et al. 2015). ATC's loco-regional invasiveness leads to severe symptoms such as dyspnoea, dysphagia, stridor and pain, and consequently impair vital thyroid function. Thus, it must be determined by an expert medical team having expertise in the field of thyroid cancers containing oncologists, endocrinologists, radiotherapists, surgeons and pathologists (Molinaro et al. 2017). The ATC cells are specified in the forms of giant, spindle-shaped and squamoid, along with major mitotic index, hemorrhage, necrosis and vascular invasion (Ragazzi et al. 2014). It is thought that advanced accumulation of hits to multiple oncogenic signaling pathways leads to ATC (Reddi et al. 2015). The most prominent gene and signaling pathways involved in ATC are respectively BRAF and RAF-BRAF-MEK-ERK. Unlike PTC and FTC, in ATC, the differentiated characteristics of tumors are impressively reduced (Wiseman et al. 2007) including declining thyroglobulin production and abundant effort to absorb iodine ions, and expanded aggressive cancer activities (Sherman et al. 2011).

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2. Pathogenesis of ATC

There are varius growth patterns of ATC to illustrate containing the pleomorphic, spindle and squamoid morphologies. In every ATC tumor, one of mentioned patterns may be predominant or demonstrate mixed feature of two or three patterns. All patterns possess the specific features of behavior like numerous mitotic forms and atypical mitoses and giant cells, wide necrosis with inflammatory infiltrates and sometimes osteoclast-like giant cells and rarely neoplastic bone and cartilage all around tumor (Keutgen et al. 2015). However, histopathologic growth patterns and histo- and cytopathology in this cancer are not likely related to patient prognosis (Smallridge et al. 2012; O'Neill et al. 2013). In ATC, thyroid is not able to generate thyroglobulin and due to the lack of specific tumor markers, its cells do not respond to thyroid-stimulating hormone (TSH) suppression. Moreover, the cells in this cancer can not uptake iodine, so radioiodine is not indicated (Molinaro et al. 2017).

3. Stages of ATC

With respect to invasive status, ATC is classified to IV

stages: IVA characterizes all the tumors that are restricted to the gland, IVB describes ATC with mass extrathyroidal development and in stage IVC the tumour is able to broaden to other sites (Smallridge et al. 2012).

Based on the degree cancer, multimodal therapy containing surgery, radiotherapy and/or chemotherapy is considered. However, most patients arrive at medical centers in the progressive stages. Due to the lack of inadequate studies and precise data and also the resistance of this cancer to standard treatments, the patients affected by ATC are suitable candidates for innovative and novel therapies such as gene targeting (Simões-Pereira et al. 2019).

4. Risk Factors

It is thought that the dedifferentiation of follicular cells leads to ATC which is associated with the decrease of the iodine levels, consequently goiter. Sufficient iodine leads to diminish tumor mass in ATC (Nagaiah et al. 2011; Keutgen et al. 2015). Also, the type B blood group is one of considerable risk factors which is related to ATC. The best prognosis for this cancer is in patients younger than 50 years old (Garcia-Rostan et al. 2015).

5. Line Treatment

With respect to the American Thyroid Association (ATA) guidelines, first-line treatment contains surgical resection and beam radiation being the two fundamental ways to control this cancer. However, both methods may be used simultaneously. Second-line treatment includes targeting genes, proteins or signaling path ways (Glaser et al. 2016). Therefore, new therapies are dedicated to this issue and a variety of treatment methods are being developed.

6. Current Treatments

There is a multimodal approach to treat ATC containing surgery, external beam radiotherapy along with chemotherapy treatment and/or palliative care (Fagin and Wells. 2016). In spite of multi-modal methods to treat, the prognosis of this cancer is negligible. With respect to the destruction of single-agent chemotherapy, more than one drug like Docetaxel, Pegfilgrastim, Doxorubicin, Cisplatin and Paclitaxel in combination is utilised for patients suffering from ATC. Second-line treatments contain various targeted therapies like anti-angiogenic drugs, and agonists, tyrosine kinase inhibitors, and multi-kinase inhibitors being able to target BCR-ABL, mTOR and BRAF (Saini et al. 2018). In this paper, we are focused on the related inhibitors effect on ATC induced by BRAF and Ras-Raf-MEK-ERK mutations.

7. Mutations

The dedifferentiation process in ATC is related to gains and eliminations in the multiple chromosomal areas leading to multiple occurrences comprising disturbances in cell cycle phases and signal transduction pathways (Kitamura et al. 2000; Hunt et al. 2003; Kadota et al. 2003). It is shown that many patients suffering from ATC is associated with a BRAF mutation. BRAF positive ATC has low survival than those of BRAF negative persons (Rashid et al. 2019). In this paper we specially focused on BRAF and Ras-Raf-MEK-ERK as inevitable targets.

8. BRAF Gene

The BRAF or v-raf murine sarcoma viral oncogene homolog B1 is a gene placed on long arm of chromosome 7 (7q34) and encodes a protein known as 18-exon cytoplasmic protein; a serine/threonine protein kinase (B-Raf). B-Raf is employed by the cell membrane as soon as growth factors ways simulation (Davies et al. 2002; Dhomen and Marais. 2007). The BRAF gene plays the substantiate role in the cellular processes of proliferation, differentiation, and apoptosis (Croce et al. 2019).

As well as MAPK kinase kinase (MAPKKK) such as CRAF and ARAF, BRAF is activated by GTPases proteins downstream from membrane receptors like KIT or EGFR (Epidermal Growth Factor Receptor) even if various types of stimuli could lead to its activation (Ottaviano et al. 2021). Various variant products of the BRAF have showen the silencing and activating of the RAS/MAPK pathway. Furthermore, a rising in protein expression or activity respectively leads the distribution of the Ras–MAPK pathway and to several developmental diseases and cancer such as ATC (Hussain et al. 2015).

BRAF protein is composed of three conserved regions (CRs) respectively with the names of CR1, CR2 and CR3. CR1 consists of a RAS-binding domain (RBD) plus a cysteine-rich domain, CR2 is a serine/threonine-rich domain including a 14-3-3 binding site and CR3 contains the catalytic serine/threonine protein kinase domain (Aramini et al. 2015). In order to be effective, BRAF dimerizes to constitute a complex with MEK, assisted by 14-3-3 proteins, playing a role in both active and inactive phases of BRAF signaling (Park et al. 2019). As soon as activated, BRAF can phosphorylate mitogen-activated protein kinase/extracellular signal-regulated kinase ERK kinase (MEK) leading to phosphorylate the last effectors of the pathway namely extracellular signal-related kinases 1 and 2 (ERK1/2) (McCubrey et al. 2007). Then they dimerize and translocate into cell nuclei leading the activation wide range of transcription factors such as c-Myc and c-Jun via phosphorylation. The last purposes of this signalling pathway in physiological situations are the regulation of cell cycle development and control of apoptosis (Ottaviano et al. 2021).

9. BRAF Mutation and RAF-BRAF-MEK-ERK Pathway Role

The mutation in the BRAF gene in thyroid gland leads to particularly PTC and ATC. BRAF mutation is divided into RAF non-V600 mutations, RAF fusions, and RAF deletions and classical V600E mutations. BRAF mutation results in hyperactivating the BRAF protein and subsequently hyperactivating MAP kinase pathways leading to tumorigenesis. Due to the location of MEK protein downstream in the MAP kinase signalling pathway, the mutation is able to hyperactivate it. One of the most considerable mutations with BRAF having more than 90% occurance in patients is BRAF V600E mutation (Podolski et al. 2019). At this point during mutation, thymine is replaced by adenine at position 1799 on exon 15 leading to glutamine amino acid instead of valine at residue 600. It is a prevalent mutation in thyroid tumors with high differentiation leading to boost progressive dedifferentiation to ATC. In ATC, Non-V600 mutations are able to upregulate or downregulate the kinase function associated with wild type BRAF. Oncogenesis can happen in such cases: through the cessation of kinase activity similar to V600E mutations, through raising dimerization/transactivation of side-by-side promoters, CRAF, resulting in hyperactivation. These mutations cause to activate BRAF and subsequently prevent ERK-mediated feedback (Long et al. 2014).

The mutated BRAF V600E is a stably activated kinase and is able to phosphorylate its downstream targets like ERK and MEK, and it is associated with more aggressive forms containing significant tumor mass, lymph node or extrathyroidal metastasis leading to unfortunately poorer outcomes (Ferrari et al. 2020). The sequence of the cascade RAF-BRAF-MEK-ERK is responsible for activating the Thyrosine kinase receptors in order to control cell differentiation and proliferation normally. The mutation of the BRAF gene leads to the surge in the activation of MEK and ERK and subsequently excessive cancer cell proliferation. Hence, the MAPK pathway is a suitable target for potential inhibitors.

10. Targeting (Inhibitors)

The BRAF-inhibitor results in declining tumor cell proliferation by way of decreasing the activation of MEK and ERK which were hyperactivated by the BRAF V600E mutation (Croce et al. 2019). Aim of this paper is to portray targeting ways leading to significant gains in reducing ATC-cells.

11. Targeting Ras-Raf and BRAF (V600E)

Ras at the first of cascade of MEK/ERK (Ras/RAF/MEK/ERK) signaling is a straight key effector in oncogenic Ras mutants and outstanding target of oncogenic mutations. The studies show that RAF is the appropriate target for drugs in order to inhibit and treat cancer development (Podolski et al. 2019). Dabrafenib is an effective powerful inhibitor of the BRAF mutation in cancer cells applied in the first line treatment of unresectable or metastatic melanoma. This inhibitor is also used in patients affected by brain metastases even after failure of local treatment (Bowyer et al. 2015). This inhibitor is able to interrupt the MAPK pathway and diminish ERK function (Hauschild et al. 2012). Treatment with Dabrafenib alone can lead to the paradoxical activation including more cancer cell survival and its development in keratoacanthomas, hyperkeratosis and squamous skin cancers in patients who are exposed to a BRAF inhibitor (Heidorn et al. 2010). Dabrafenib is able to decrease MEK/ERK phosphorylation and prevent the viability of BRAF mutated cells via arresting G0/G1 phase. After the treatment with the help of Dabrafenib singly, the phosphorylation of MEK is risen anew then VEGF is soared (Kurata et al. 2016). Dabrafenib in combination with a MEK inhibitor leads to hamper the increase of MAPK signaling activity and decline the side effects (King et al. 2013). Except Dabrafenib, Vemurafenib and Encorafenib are first-generation RAF inhibitors used to cure patients affected by BRAF V600E mutation (Degirmenci et al. 2020). Encorafenib is a BRAF inhibitor with more prolonged pharmacodynamic function than other certified BRAF inhibitors. Encorafenib plus (BRAF inhibitor) Binimetinib (MEK inhibitor) has impressive effects on patients suffering from BRAF V600-mutated metastatic thyroid cancer with resistance to radioiodine treatment (Shin et al. 2020). Sorafenib is an oral small multikinase inhibitor of the serine/threonine-kinases (c-RAF and BRAF) with the capability of suppressing Raf/MEK/ERK signaling pathways, blocking the vascular endothelial growth factor receptor 2 (VEGFR2) and VEGFR3, inhibiting respectively platelet-derived growth factor receptors (PDGFR), FLT3, Ret, and c-KIT (Ziogas and Tsoulfas, 2017). In addition, it has been illustrated to result in apoptosis in different human tumor cell lines (Rahmani et al. 2005). This inhibitor has a beneficial effects to treat ATC at a low frequency (Saini et al. 2018). Vemurafenib is an orally selective low weight molecule to inhibit the V600 BRAF mutation (da Rocha Dias et al. 2013). The clinical experiments show that Vemurafenib has low toxicity and quick response in progressive melanoma patients arising from the BRAF V600 mutation (Kim and Cohen, 2016). Monotherapy with the help of Vemurafenib is impressive in Non-small-cell lung carcinoma (NSCLC) arising from BRAF V600 mutations (Mazieres et al. 2020). This inhibitor can also lead to block BRAF kinase pathways successfully for a short time in ATC (Marten and Gudena, 2015). Vemurafenib in combination with MEK inhibitors has more success in treatment (Degirmenci et al. 2020).

The inhibitors mentioned usually affect monotherapy in a short time and drug resistance will rise and the efficacy

decline. Reactivation of cancer cells during the treatment process recurs via two distinct ways: the first in elevating the level of active Ras in the cell leading to paradoxical activation of ERK signaling and the second is alternative splicing BRAF(V600E) sequence in order to produce variants along with truncated N-terminus leading to promote BRAF(V600E) homodimerization and reduce the drug influence. Significantly, the cancer cells affected by these drugs become addicted RAF inhibitors leading to finally postpone the development of resistant cancers (Degirmenci and Wang, 2020).

The paradoxical influences of RAF inhibitors not only leads to enhance effective therapeutic efficacy but also leads to generate other malignant cancer cells. To overcome their side effect, second-generation RAF inhibitors known as pan-RAF inhibitors and paradox breakers were expanded. pan-RAF inhibitors includes RAF265, LXH254, BAL3833, BGB283, TAK632, TAK580, CCT3833 and LY3009120, and PLX8349 is an example of paradox breakers (Degirmenci and Wang, 2020). The pan-RAF inhibitors are able to block both promoters via dimerization with the same affinity whereas the paradox breakers are able to generate αC helix-out conformation leading to hamper the dimerization. MEK and ERK are also substantial targets in Ras/RAF/MEK/ERK signaling pathway.

12. MEK and MEK Inhibitors

MEK is located in the downstream of both RAS and RAF in Ras/RAF/MEK/ERK signaling pathway (Falchook et al. 2012). MEK inhibition as a single-drug therapy is able to upregulate the protein machinery expression being essential to uptake iodine in the ATC cell lines of human (ElMokh et al. 2019). MEK1/2 play substantial mediating roles in signal transduction inside of the cell and have crucial oncogenic function to in occurrence of wide range of tumors, and thus they are noteworthy targets to treat the cancers (Zhao and Adjei, 2014). Trametinib is an orall inhibitor with the reversible selective allosteric capability for both of MEK1 and MEK2 (Sanchez et al. 2018). Also, Trametinib is able to inhibit cell viability via downregulation of ERK phosphorylation (Kurata et al. 2016). However, Trametinib is approved for monotherapy, latest clinical researchs recommends the positive impressive influences of Trametinib in combination with Dabrafenib (Sanchez et al. 2018). Trametinib can even cease the development of other cancers such as cutaneous squamous-cell carcinoma (cSCC) (Flaherty et al, 2012). The usage of Trametinib with Dabrafenib simultaneously is an advanced promising targeted treatment for the patients suffering from BRAF V600E–mutated ATC leading to positive prolonged responses with extended survival with the capability of controllable toxicity. This is the first regimen to illustrate strong clinical function in BRAF V600E–mutated ATC (Subbiah et al. 2018). Cobimetinib is one of recent MEK inhibitor to treat BRAF or KRAS mutant cancer cells proven in vivo human mutant xenograft tumors (Hoeflich et al. 2012). Both the European Union and the FDA approved Cobimetinib in combination with Vemurafenib to treat progressive stage BRAFV600E (Sanchez et al. 2018) and considerable therapeutic effects during ATC treatment (Green et al. 2017). However, it is emphasized that Cobimetinib should not be used alone (Sanchez et al. 2018). The experiments show that Cobimetinib and Trametinib are able to suppress BRAF(V600E) as single agents or in combination with RAF inhibitors (Welsh and Corrie, 2015; Cheng and Tian, 2017). Selumetinib is another orall inhibitor with selective allosteric capability for both of MEK1 and MEK2 (Banerji et al. 2010). The clinical researches demonstrate that Selumetinib is an appropriate drug to inhibit the metastatic differentiated thyroid tumors with resistance to radioiodine concentration. The incapacity of iodine concentration in the thyroid cancers is associated with BRAF and RAS mutations leading to activate the function of MAPK signaling (Chakravarty et al. 2011). Selumetinib can more sensitize the differentiated thyroid cancer cells in patients in order to treat it effectively via radioiodine (Ho et al, 2013). Trametinib and Selumetinib are able to hamper MEK function in the pathway of MAP kinase signaling and subsequently reduce adverse effects associated with paradoxical ERK activation in comparison with monotherapy through BRAF inhibitors (Podolski et al. 2019).

Pimasertib is a selective small-molecule MEK inhibitor of MEK1/2 with powerful anti cancer activity alone or in conjugation with other compounds such as the PI3K inhibitor SAR245409, gemcitabine and the HDM-2 inhibitor SAR405838 in the cell lines and xenograft models (von Richter et al. 2016). Pimasertib plus the BRAF inhibitor PLX4032 is able to activate apoptosis process in the cell lines induced by BRAF-mutated human. Resently, Pimasertib is expanded in order to treat the solid and hematologic cancers (Scheible et al, 2017). Binimetinib is a new orally available small molecule MEK inhibitor of cell proliferation in mutant B-Raf and Ras cell lines and has the anti-tumor capability in the various cancers (Lee et al. 2010). The clinical research illustrates that Binimetinib in combination with FOLFOX is able to inhibit colorectal cancer in patients who previously advanced on standard therapies (Cho et al. 2017). Binimetinib in combination with Encorafenib treats unresectable of Metastatic melanoma induced by BRAF V600 mutation (Subbiah et al. 2020) whereas no data about its effect on ATC is available.

13. ERK and ERK Inhibitors

ERK is an appropriate site in the Ras/RAF/MEK/ERK signaling pathway which is why the upstream molecule RAF has little effectors except MEK. It shows that ERK is only the substrate for the upstream molecule RAF and the only activator to stimulate various downstream substrates. ERK1/2 inhibitors are able to inverse the abnormal function of the Ras/RAF/MEK/ERK signaling pathway due to

upstream mutations containing RAS mutations. ERK is only downstream and negative inhibitor of RAF (Liu et al. 2018). Besides, research indicates that appropriate differences in the spatio-temporal activation of ERK leads the creation of various signaling outputs and subsequent control of biological responses. In addition, interaction between ERK and other signaling pathways results in vitally determining cell fate (Ramos. 2008). Considerably, optimal ERK signaling is necessary to develop cancer cells whereas increasing signaling led to toxicity and subsequently death in them. The higher ERK signaling arises in drug-resistant cancer cells while the drug-sensitive cancer cells are less signaling. The drug treatment can attenuate the ERK signaling in the drug-resistance cells leading to create appropriate condition for the cancer development whereas drug holiday or drug withdrawal result in preventing their expansion (Seghers et al. 2012; Wang et al. 2018). In spite of the lack of paradoxical influences of ERK inhibitors, they have limited efficacy to treat associated with cascade blockage in the normal cells (Welsh and Corrie, 2015; Cheng and Tian, 2017). However, another research illustrates that the synergistic inhibition of MEK and ERK leads to the hampering of resistance and persistent resistance induced by MEK (Merchant et al. 2014). With respect to Thomson Reuters data, BVD-523 and GDC0994 are two ERK inhibitors with complete clinical trial. Recently, however, a few ERK inhibitors have been reported, including GDC-0994, RG-7842, CC-90003, KO-947, LTT-462 and LY-3214996, all of which are in the pre-clinical study phase (Liu et al. 2018). Although, the monotherapy with ERK inhibitors is still being researched (Sanchez et al. 2018), it probably seems that the combination of inhibitors mentioned with other types of inhibitors leads to accurate and effective results.

14. Conclusion and Remarks

The treatment of patients affected by ATC is very challenging. ATC is a rare disorder and very invasive with lethal result so far. This article specifically focused on ATC induced by mutations in BRAF V600E and Ras-Raf-MEK-ERK signaling pathways and their inhibitors. After identifying ATC, targeting by inhibitors can be one of the best suitable treatments with maximum therapeutic benefits without the need for surgery and radiotherapy or as a complementary therapy. Monotherapy trials with appropiate inhibitors should be pursued to recognize for more effective therapies and eradicate the cancer cells. However, to ensure effectiveness for prevention of cancer recurrence and to limit the mechanisms of resistance, it seems necessary to use more than one inhibitor in targeting therapy. On the other hand, producing new inhibitors with less side effects in targeting therapy will be the ray of hope to prolong survival in patients affected by ATC and be a good alternative to surgery and radiotherapy in the future. However, some data obtained are still based on case reports, and further clinical studies are required to create comprehensive treatment.

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Potential Conflict of Interest

The author has no conflicting financial interests.

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