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Advances in ALK Targeted Therapy for Neuroblastoma

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Abstract

Significant advances have been made to understand the association between ALK genetic aberrations and disease prognosis in neuroblastoma. ALK targeted therapies are evolving quickly and several randomized controlled trials of ALK inhibitors are underway or nearing completion in adult cancers. Ongoing research will bring new challenges and newer technologies to fully define the pathogenic and prognostic alterations, to stratify the risk of recurrence or progression, and to develop optimal monitoring and treatment strategies in this malignancy.

Keywords: ALK; Neuroblastoma; Drug treatment

Introduction

Neuroblastoma is the most common extracranial solid tumor originating from the sympathoadrenal lineage of the neural crest. It has extensive pathologic and molecular heterogeneity which decides the significant clinical diversity from spontaneous regression to highly-aggressive and drug resistant metastatic disease. Although multimodal chemotherapy/radiotherapies/immunotherapy significantly improves patient survival during the last few decades, some patients will continue to relapse and die of this malignancy because of de novo or acquired drug resistance, especially for high-risk neuroblastoma patients [1,2].

Over the last decades, concerted efforts have been made to identify oncogenic alterations in subsets of neuroblastoma, such as molecular alterations of MYCN, anaplastic lymphoma kinase (ALK), paired-like homeobox 2B (PHOX2B), etc. [3-6]. Next-generation sequencing-based genomic profiling identified the most frequent alterations including MYCN (26.5%), ALK (17.8%), ATRX (6.5%), CDKN2A (4.8%) and RPTOR (4.8%) in 230 neuroblastoma patient samples [7]. Both ALK and MYCN genes are located in chromosome 2p, a chromosomal alteration identified as a statistically significant prognostic factor [8]. It has been shown that ALK and MYCN drive tumor malignancy cooperatively. Activation of ALK increases the expression of MYCN by enhancing the activity of the MYCN promoter and stabilizing MYCN protein likely via activation of AKT and ERK pathways [9-11]. *In vivo*, compared to ALKF1174L and MYCN alone, co expression of these two oncogenes leads to the development of neuroblastoma tumors with earlier onset, higher penetrance and enhanced lethality [10,12,13]. In our recent study, neuroblastoma cells harboring both ALKF1174L mutation and MYCN amplification showed less responsive to an ALK inhibitor, crizotinib, comparing to other variants [14]. Understanding those tumor-specific, oncogenic driver mutations would provide further insight into the biology of this disease and transformed our treatment strategy into the era of precision medicine.

ALK Variants in Neuroblastoma

Among all those identified oncogenic mutations, ALK is one of the well-studied druggable molecular targets. ALK was first discovered in 1994 as a fusion protein with nucleophosmin (NPM) in a subset of anaplastic large-cell lymphomas (ALCLs) as a result of t (2; 5) (p23; q35) chromosomal translocation [15]. In 2007, ALK gene rearrangements were reported in a subset of patients with non-small cell lung cancer (NSCLC) [16]. Since then, ALK mutations have been regarded as oncogenic mutations. More mutations of ALK gene have been reported in different cancer types, including NSCLC, inflammatory myofibroblastic tumors, colon cancer, renal cell carcinoma, breast carcinoma and esophageal cancer [17]. In 2008, several groups discovered ALK mutations, including germline missense mutations and somatically acquired mutations, in high-risk neuroblastoma patients [18-20]. They also found that mutated kinases were autophosphorylated and displayed increased kinase activity compared with the wild-type ALK.

So far, more than 35 ALK variants have been detected in neuroblastoma, predominantly point mutations [21], with fewer cases of truncated extracellular domain [22,23] and BEND5-ALK fusion protein [7]. ALK mutations are found in almost all cases of familial neuroblastoma (<2% of all neuroblastoma) [24]. There are three most common pathogenic variants ALKR1275Q, ALKG1128A and ALKF1174L identified in familial neuroblastoma patients, among which ALKR1275Q accounts for 45% of ALK germline mutation [4,18]. ALK mutations have also been reported in about 6-10% of sporadic neuroblastoma cases [25]. 12 different ALK active mutations have been reported in sporadic neuroblastoma, including two most common ALK variants, ALKF1174L and ALKR1275Q [26]. Most of these pathogenic variants are found within the tyrosine kinase domain of ALK and cause constitutive autophosphorylation and activation of the ALK protein and downstream cellular pathways, including the MAPK and RAS-related protein 1 signal pathways [9,27]. The PI3K (phosphatidylinositol 3-kinase)/Akt [10] and the JAK/STAT (Janus activated kinase/signal transducer and activator of transcription) pathways [28].

ALK Targeted Agents in Neuroblastoma

In 2011, crizotinib (PF-02341066, Xalkori) became the first FDA-approved ALK inhibitor for ALK-positive NSCLC based on strong

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clinical response data [29]. It is an oral small-molecule tyrosine kinase inhibitor, originally developed as a c-MET inhibitor and later found an inhibitor for ALK phosphorylation [30,31]. *In vitro* studies demonstrated that crizotinib is potent in neuroblastoma cell lines with ALK amplification or the R1275Q mutation, one of the most common ALK variants in neuroblastoma [32]. Whereas cells bearing ALKF1174L mutation are relatively crizotinib-resistant [18,19,33,34]. *In vivo*, crizotinib treatment causes complete and sustained regression of xenografts with ALKR1275Q mutation, but it has limited effects on the growth of ALKF1174L-positive tumors [21]. Crizotinib has been tested in Phase I clinical trial for the treatment of pediatric solid tumors and it is currently being tested in a subset of neuroblastoma bearing ALK mutations and rearrangements [35].

Same as other tyrosine kinase inhibitors, crizotinib invariably loses its potency and drug resistance emerges after initial successful crizotinib treatment. Crizotinib resistance may develop in multiple tumor types as a consequence of secondary mutations in the ALK tyrosine kinase domain, the activation of other bypass pathways, or amplification of the ALK locus [36,37].

Epithelial-mesenchymal transition (EMT) also contributes to resistance to crizotinib in lung cancer cells [38], especially for second-generation ALK inhibitors [39]. In neuroblastoma, ALKF1174L mutation serves as a common cause of resistance to crizotinib, due to the increased ATP-binding affinity of this mutant [40]. ALK F1174L mutation also causes resistance to other ALK inhibitors. Acquired resistance to TAE684 and LDK378 was observed in ALKF1174 mutant human neuroblastoma cells, which is associated with AXL activation and induction of EMT [41]. Some other mechanisms could also regulate the sensitivity of ALK inhibitors in neuroblastoma. A recent study with genome-wide microRNA profiling identified that microRNAs, miR-424-5p and miR-503-5p, were involved in regulating ALK expression, which may serve as potential therapeutic tools in ALK dependent neuroblastoma [42]. In some cases, crizotinib resistance may also arise from pre-existing minority cell populations with drug resistance due to intratumor heterogeneity in neuroblastoma tumors. Yan et al. [43] identified a subpopulation of neuroblastoma cells that are insensitive to the ALK kinase inhibitor. These cells have Schwann cell–like features, possess unique signaling profiles, but express the undetectable level of ALK. Treatment of SK-N-SH with an ALK kinase inhibitor TAE684 results in the outgrowth of the S-type cells. These TAE684-resistant S-type cells are also believed to protect N-type cells against the apoptotic effect of an ALK kinase inhibitor through upregulating prosurvival signaling [43].

In order to settle crizotinib resistance, several other ALK inhibitors, including ceritinib, brigatinib (AP26113), alectinib, lorlatinib (PF-6463922), ensartinib (X-396), entrectinib (RXDX- 101), and belizatinib (TSR 011), have been developed in clinical use for adult patients [44,45].

Among those ALK inhibitors, only ceritinib and lorlatinib have been tested in clinical trials for pediatric patients. Ceritinib was approved by FDA in 2014 for the treatment of patients with ALK-positive lung cancer who relapse after first-line therapy [46]. It overcomes some crizotinib- resistant mutations, but ALK tumors harboring the ALKF1174L mutation still exhibit resistance to ceritinib [47,48]. Lorlatinib, as a selective next-generation ROS1/ALK inhibitor, has high potency across ALK variants, ALKR1275Q, ALKF1174L and ALKF1245C mutations. It induces complete tumor regression in both crizotinib-resistant and crizotinib-sensitive neuroblastoma xenograft models, as well as in patient-derived xenografts [45]. A new ALK/IGF1R inhibitor AZD3463 was designed by AstraZeneca to overcome the acquired resistance to crizotinib.

This new drug suppressed cell proliferation of neuroblastoma cell lines with wild type ALK as well as ALK activating mutations (ALKF1174L and ALKD1091N) by blocking the ALK-mediated PI3K/AKT/mTOR pathway. In addition, AZD3463 also exhibited significant therapeutic effects on the growth of the NB tumors bearing an ALKF1174L mutation in orthotopic xenograft mouse models [47]. Most recently, A novel ALK inhibitor alectinib (5-chloro-2,4-diaminophenylpyrimidine) has been tested in neuroblastoma preclinical models and showed substantial inhibitory effects against tumors with ALK mutations, including ALKL1152R, ALKF1174L and ALKD1091N [49,50]. Although the new generation ALK inhibitors overcome crizotinib-resistant ALK mutations, patients almost invariably relapse. Genotype assessment of repeat biopsies from ALK-positive patients progressing on various ALK inhibitors revealed that only a minority of ALK-positive patients (~20%) developed ALK resistance mutations on crizotinib, while ALK resistance mutations were present in over one-half of patients progressing on second-generation ALK inhibitors. Also, the spectrum of ALK mutations was different following second-generation ALK inhibitors compared to crizotinib [39]. New and alternative approaches are required to tackling drug resistance against ALK inhibitors.

Combined Therapy with ALK Inhibitors

One of the mechanisms by which cancer cells evade kinase inhibitor-induced apoptosis is to switch to alternative signaling pathways. Combinatorial approaches to inhibit multiple kinases could be a therapeutic possibility to reverse drug resistance acquired from activation of bypass pathways. To overcome the drug resistance secondary to ALK inhibitors, Krytska et al. [51] combined crizotinib with the chemotherapeutic agents and showed increased cytotoxic effects comparing to crizotinib or chemotherapy alone *in vitro*. Combined therapy also restored sensitivity in preclinical models harboring ALK aberrations (both mutation and amplification). Hypoxia regulates tumor cell proliferation, migration and invasiveness through the expression of a group of transcription factors called hypoxia-inducible factors (HIFs) [52,53]. It has been demonstrated that ALK specifically regulates HIF-1α expression under hypoxia conditions in both ALCL and NSCLC [54]. Topotecan, especially daily metronomic topotecan, induces oxidative stress and down-regulates HIF-1 alpha expression in cancer cells [55-57]. In our recent study, single-agent crizotinib showed limited anti-tumor activity in ALKF1174L-mutated neuroblastoma xenograft models, however when combined with topotecan, significantly delayed tumor development was achieved. This anti-tumor activity was achieved through targeting of this hypoxia related pathway. In addition, relapsed tumors remained responsive to combined therapy [14]. Synergistic antitumor activities have also been observed when combining ALK inhibitor ceritinib with a dual inhibitor of cyclin-dependent kinase CDK4 and CDK6 [58] or with an MDM2 inhibitor NVP-CGM097 which targets p53 for proteasome-mediated degradation [59].

Future Directions – Precision Medicine in Neuroblastoma

Targeted agents for different ALK variants improved clinical outcomes over the last few years, with newer agents rapidly entering clinical practice. However, ALK genetic alterations are not routinely evaluated in neuroblastoma standard clinical practice. Newer technologies are being introduced to fully define the pathogenic and prognostic impact of ALK alterations in neuroblastoma, as well as the emerging importance for therapeutic purposes. Lodrini et al. [60]

established droplet digital PCR (ddPCR) protocols for MYCN and ALK copy number status in cell-free neuroblastoma-derived DNA isolated from plasma of neuroblastoma patients. They accurately discriminated between MYCN and ALK amplification, gain and normal diploid status in conditioned culture medium, mouse plasma from xenograft models, as well as NB patient plasma [60]. Tucker et al. [61] used ALK immunoassays to detect ALK and phosphorylated ALK and revealed a quantitative difference in on-target pharmacodynamic changes between a first- and second-generation ALK inhibitor, crizotinib and ceritinib, which will potentially become a valuable tool in preclinical and clinical evaluation in defining active doses and optimum treatment schedules [61].

Precision medicine has become a new model of health care aimed at tailoring therapies to an individual's genetic profile. As technologies and therapies improve, retrospective and prospective studies of cohorts of neuroblastoma patients are being required to incorporate genetic analyses into clinical practice beyond diagnostic purposes. A retrospective study by Padovan-Merhar et al. [62] defined the frequency of genomic alterations by gene panel sequencing in neuroblastoma patients, both at diagnosis and after chemotherapy and showed a higher frequency of ALK mutations in relapsed disease than at diagnosis. Suspected driver ALK variants were present in 3/43 (7.0%) of samples at diagnosis, 7/41 (17%) post-treatment samples, and in 11/54 (20%) of samples at relapse. The same trend was observed in some other tumor-related genes, particularly for the alterations in the RAS/MAPK pathway, in relapsed high-risk neuroblastoma [62].

Conclusion

A better understanding of the switch to alternative signaling pathways will lead to strategies to bypass drug resistance. This proof of principle may also apply to other genetic alterations such as MYCN, NRAS, NTRK2/TrkB, etc. Given the growing number of genetic alterations detected, small sequencing panels that focus on a limited number of genes may not be sufficient, especially in highly heterogeneous neuroblastoma tumors. Further improvements in next-generation sequencing technologies are expected to allow the evaluation of genetic variants across the entire genome, which is also a more straightforward strategy for mapping mutations.

The molecular profile of each individual patient at different stages of treatment will inform physicians the detailed genetic condition and enable personalized targeted therapeutic interventions in specific subsets of neuroblastoma patients.

Disclosure of Potential Conflicts of Interest

Dr. Sylvain Baruchel participates in consulting activities for NeoMed Inc. (Montreal, Quebec).

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