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N-MycInhibition: Advances in Neuroblastoma Treatment

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Abstract

Neuroblastoma (NBL) is one of the most common solid tumors and around 15% of cancer mortality in children. Amplification of the *N-Myc* proto-oncogene is strongly correlated with advanced disease and poor clinical outcome in NBL. Recent studies described that ubiquitin-specific protease 7 (USP7; also known as HAUSP) interacts with N-Myc, induces deubiquitination and subsequent stabilization of N-Myc that in-turn potentiates N-Myc function, and treatment with the HAUSP inhibitor (P22077) blocked such effects.

Keywords

Neuroblastoma, N-Myc, PI3K/mTOR, PARP1, PD-L1,HAUSP

1. Introduction

Neuroblastoma (NBL), the most common solid tumor of childhood, is originated from the neural crest cells of the developing sympathetic nervous system [1]. It represents 8% - 10% of pediatric tumors and accounts for 15% of all pediatric cancer deaths [2] [3] [4]. In the recent years, considerable progress has been made in the treatment effect of NBL including chemotherapy, radiotherapy, surgical resection and hematopoietic stem cell transplantation; however the 5-year overall survival (OS) is still less than 50% in high-risk NBL [4] [5].

The genetic feature most consistently associated with treatment failure is an amplification of the *N-Myc* proto-oncogene, which is strongly correlated with advanced disease and poor prognosis [6] [7] [8] [9] [10]. Generally, amplification of *N-Myc* occurred in neuroblastoma based on the mechanisms involving double minutes (dmin) or homogeneously staining regions (hsr) [11]. Expression of N-Myc is associated with accelerated proliferation, migration, invasion

and metastasis [3] [12] [13] [14] [15]. Consistence with these evidences, *N-Myc* transgene can induce tumor formation in transgenic mice [16], and *N-Myc*-knockout mouse shows embryonic lethality [17] [18] [19], whereas the *Nes-Cre*-driven conditional knockout of *N-Myc* has a decrease in cerebellar and cerebral cortex mass due to defective cellular proliferation [20].

At the early stage of NBL, it is often clinically unrecognized [21]. The primary tumor usually occurs in the abdomen (60%), but neuroblastoma children present with metastasis more than 50% at diagnosis [2]. NBL metastasis is usually present in the bone marrow (70.5%) or the skeleton (55.7%); patients may also present with metastasis in the lymph nodes (30.9%), liver (29.6%), or intracranial and orbital sites (18.2%) [22]. Recently, Yue Z-X. *et al.* demonstrated that clinical outcome was poorer in NBL patients metastases to bone marrow with *N-Myc* amplification than in those without amplification [23].

2. Results

2.1. PI3K/mTOR/N-Myc Inhibition

Both C-Myc and N-Myc contribute to the regulation of VEGF and angiogenesis. Like C-Myc, N-Myc is stabilized by activation of phosphatidylinositol 3-kinase (PI3K) [24], and inhibition of PI3K and mTOR (mammalian target of rapamycin) leading to reduced secretion of VEGF and decreased levels of N-Myc protein [25] [26] [27]. Moreover, Chanthery Y.H. *et al.* demonstrated that a clinical PI3K/mTOR inhibitor, NVP-BEZ235, decreased angiogenesis and improved survival on N-Myc dependent mechanism in both primary human (highly pretreated recurrent N-Myc-amplified orthotopic xenograft) and transgenic mouse models for N-Myc-driven neuroblastoma, suggesting that NVP-BEZ235 should be tested in children with high-risk, N-Myc-amplified neuroblastoma [28].

2.2. PARP1/N-Myc Inhibition

Poly (ADP-ribose) polymerase (PARP) is involved in a number of cellular processes such as DNA repair, genomic stability, and programmed cell death in the response to numerous endogenous and environmental genotoxic agents [29] [30] [31]. Survival studies of PARP knockout ($PARP^{-/-}$) mice after γ-irradiation showed that, $PARP^{-/-}$ mice died within 10 days post-irradiation compared to wild-type controls, those remained apparently healthy [29]. Recently, Colicchia V. *et al.* described that higher expression of PARP1 was associated with poor clinical outcome in NBL patients [32]. Moreover, PARP1 is highly expressed in N-Myc amplified and advanced stages compared to N-Myc non-amplified and lower stages in primary NBL or NBL cell lines; supporting N-Myc inhibition might be a promising developmental therapy in NBL.

2.3. PD-L1/N-Myc Inhibition

Cancer immune evasion is a major stumbling block in designing effective anticancer therapeutic strategies [33]. Cancer cells frequently produced factors such

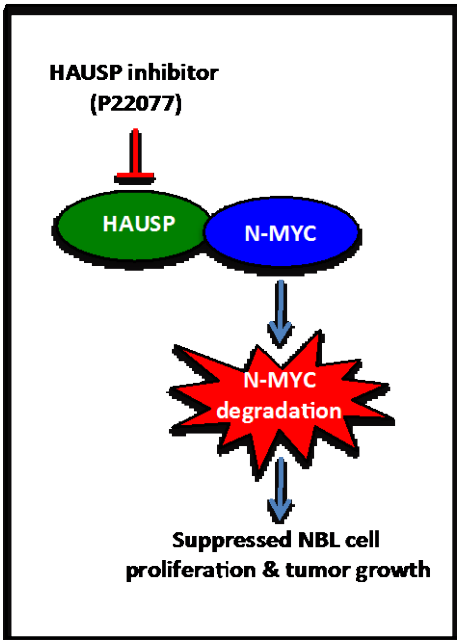

Figure 1. Schematic diagram of model shows the association between HAUSP and N-MYC, and how HAUSP inhibitor (P22077) can facilitate the degradation of N-MYC protein that in-turn suppressed Neuroblastoma (NBL) cell proliferation & tumor growth. Courtesy of Tavana O. *et al.* (modified by M.K. Hasan).

as PD-L1, adenosine, IL-10 and TGF-β that bind negative regulatory surface receptors expressed on cytotoxic T cells [34]-[39]. Tumor cells expressed PD-L1 on the surface and prevent binding of its inhibitory receptor PD-1 on T cells [40]. Targeting PD-L1 or PD-1 by mAb immunotherapies was shown to have pronounced anti-tumor activity in clinical trials [34] [35] [41] [42]. Recently, Melaiu O. *et al.* described that higher level of PD-L1 expression was correlated to unfavorable prognosis in NBL patients [43]. PD-L1 expression was observed higher with N-Myc amplification in NBL patients and cell lines. Moreover, N-Myc blockade causes suppression of PD-L1 expression in NBL; suggesting N-Myc-inhibition therapy could restore an efficient anti-tumor immunity in high-risk neuroblastoma.

2.4. HAUSP/N-Myc Inhibition

Ubiquitin-specific protease 7 (USP7; also known as HAUSP) is a ubiquitin specific protease or a deubiquitylating enzyme that cleaves ubiquitin from its substrates [44]. As ubiquitylation process (polyubiquitination) is most commonly associated with the stability and degradation of cellular proteins, HAUSP activity generally stabilizes its substrate proteins. In cancer biology, HAUSP have important role for the modulation of the stability and activity of several cellular proteins [45]-[50].

Recently, Tavana O. *et al.* found that ubiquitin-specific protease 7 (USP7 or HAUSP) directly interacts with N-Myc, and HAUSP expression induces deubi-

quitination and subsequent stabilization of N-Myc [51]. RNA interference (RNAi)-mediated knockdown of *USP7* in neuroblastoma cancer cell lines, or genetic ablation of *Usp7* in the mouse brain, inhibits stabilization of N-Myc protein, which leads to suppression of N-Myc function. Structural analysis revealed that amino acids from 281 to 345 region of N-Myc protein are necessary for this interaction.

Moreover, high expression of HAUSP in patients with neuroblastoma is associated with poor prognosis, and significantly correlates with *N-Myc* transcriptional activity. Treatment with the small-molecule inhibitor of HAUSP (P22077) suppressed cell proliferation, and the growth of xenograft tumor models in mouse derived from *N-Myc*-amplified human neuroblastoma cell lines. (See Figure 1).

3. Conclusion

Overall results suggesting that inhibition of N-Myc might have important applications for the treatment of NBL patients with *N-Myc* amplification.

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