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EDITORIAL

Combination therapies improve the anticancer activities of retinoids in neuroblastoma

Belamy B Cheung

Belamy B Cheung, Molecular Carcinogenesis Program, Children's Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, Sydney NSW 2031, Australia

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Correspondence to: Belamy B Cheung, PhD, Molecular Carcinogenesis Program, Children's Cancer Institute, Lowy Cancer Research Centre, University of New South Wales, PO Box 81, Randwick, Sydney NSW 2031, Australia. bcheung@ccia.unsw.edu.au
Telephone: +61-2-93852450
Fax: +61-2-96626584

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Abstract

Most therapeutic protocols for child cancers use cytotoxic agents which have a narrow therapeutic index, and resulting in severe acute and chronic toxicities to normal tissues. Despite the fact that most child cancer patients achieve complete remission after chemotherapy, death still occurs due to relapse of persistent minimal residual disease (MRD) which remaining after initial cytotoxic chemotherapy. Advanced neuroblastoma (NB) is a leading cause of cancer deaths in young children. Retinoids are an important component of advanced NB therapy at the stage of MRD, yet half of all patients treated with 13-*cis*-retinoic acid still relapse and die. More effective combination therapies, with a lower side-effect profile, are required to improve outcomes for NB. Fenretinide or N-4-hydroxyphenyl retinamide is a synthetic derivative of retinoic acid which works on cancer cells through nuclear receptor-dependent and -independent signalling mechanisms. Moreover, several histone deacetylase inhibitors have entered early phase trials, and, suberoylanilide hydroxamic acid has been approved for use in adult cutaneous T cell lymphoma. A number of studies suggest that retinoid signal activation is necessary for histone deacetylase inhibitor activity. A better understanding of their mechanism of actions will lead to more evidence-based retinoid combination therapies.

Key words: Retinoids; Histone deacetylase inhibitors; Combination therapies; Neuroblastoma; Fenretinide

Core tip: Neuroblastoma (NB) begins in embryonal neural crest cells, which later give rise to the sympathetic nervous system, and is caused in part by factors which arrest differentiation. *In vitro*, retinoids force susceptible cancer cells down a pathway of terminal differentiation and, have been part of the routine treatment of advanced NB for number of decades. Synergistic anti-tumour activity between histone deacetylase inhibitors and retinoids has been observed in a variety of preclinical models. This editorial note discusses some of these findings on the combination therapies for improving the anticancer activities of

retinoids in NB.

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INTRODUCTION

Neuroblastoma (NB) is a tumor of the sympathetic nervous system and the most common extracranial solid tumour in childhood[1]. NB accounts for more than 7% of malignancies in patients younger than 15 years and around 15% of all paediatric oncology deaths[2]. Some infants experience spontaneous regression, whereas older patients have maturation of their tumor into benign ganglioneuromas. However, the outcome for children with a high-risk clinical phenotype has improved only modestly, with long-term survival still less than 40%[3]. The introduction of 13-*cis*-retinoic acid (13-*cis*-RA) in the therapy of NB has improved the prognosis of this disease. Currently, the standard treatment for high risk of NB consists of myeloablative therapy followed by autologous hematopoietic stem cell transplantation and maintenance with 13-*cis*-RA for the treatment of minimal residual disease (MRD), leading to a 3-year disease-free survival rate of about 50%[4]. Retinoids are an important component of advanced NB therapy at the stage of MRD, yet half of all patients treated with 13-*cis*-retinoic acid still relapse.

Retinoid therapy in paediatric cancer

Retinoids are vital for the growth and differentiation of a variety of normal adult and embryonic tissues, and have potent antiproliferative effects on many malignant cell types[5]. Retinoids mediate their widespread effects on cells by regulating the transcription of target genes through a complex system of ligand-inducible nuclear transcription factors: The retinoic acid receptors and retinoid X receptors[6]. RA exists in several stereoisomeric forms: Predominantly *all-trans* retinoic acid (ATRA) and 13-*cis*-RA, but also as less-stable isomers such as 9-*cis* retinoic acid. In the last few decades they have been widely studied in cancer prevention and therapy because of their ability to induce differentiation of tumor cells[7]. Retinoids are successfully used for the treatment of one pediatric cancer: Acute promyelocytic leukemia. ATRA converts the PML-RAR-α fusion protein into activator of transcription and restores cell differentiation[8]. Retinoids have also been widely investigated in solid tumors, especially in NB. In a long-term study for children with high-risk NB treated on a randomized trial of myeloablative therapy followed by 13-*cis*-RA, which given after intensive therapy resulted in significant improvement in 5-year overall survival rates, regardless of the type of consolidation[9]. However, as many high-risk patients still ultimately die due to relapse of persistent MRD after initial cytotoxic chemotherapy, novel therapies effective against MDR NB are needed.

Fenretinide is an effective retinoid therapy

Retinoids are vitamin A analogues required for normal morphogenesis and maintenance of diverse embryologic and adult tissues, which act on cells by binding nuclear receptors[10]. Fenretinide or N-4-hydroxyphenyl retinamide (4-HPR) is a synthetic derivative of retinoic acid which works on cancer cells through nuclear receptor-dependent and -independent signalling mechanisms[11]. 4-HPR has a broad spectrum of cytotoxic activity against primary tumor cells, cell lines, and/or xenografts of various cancers, including NB [11-13] and has been tested in early phase clinical trials in recurrent NB [14,15]. 4-HPR was anti-angiogenic in multiple tumour types and cytopathic in some cancer cells which were resistant to other retinoids or chemotherapeutics[13]. Clinical trials have revealed that 4-HPR is a highly active therapeutic and chemo-preventive agent with minimal side-effects in NB[14]. A phase Ⅰ/Ⅱ trial of oral 4-HPR in children with high-risk, relapsed solid tumours demonstrated minimal 4-HPR toxicity, but only stable disease as the best clinical response[16].

Combination therapy improves the anticancer activity of histone deacetylase inhibitors and retinoids

Increased histone deacetylase activity is a common causal factor in human cancer that causes transcriptional silencing of tumour suppressor genes[17]. Histone deacetylase inhibitors prevent deacetylases removing acetyl groups from histone tails, thereby promoting gene transcription[18]. Several histone deacetylase inhibitors have entered early phase trials, and, suberoylanilide hydroxamic acid (SAHA) has been approved for use in adult cutaneous T cell lymphoma[19]. The histone deacetylase inhibitor side-effect profile is low when compared with cytotoxic chemotherapy[20]. Moreover, two unbiased preclinical screens identified retinoid signal activation as the most effective method of augmenting the histone deacetylase inhibitor anti-cancer signal[21,22]. Retinoic acid receptor α (RARα), and, preferentially expressed antigen of melanoma, both repressor proteins for the retinoid signal, was shown to mediate resistance to histone deacetylase inhibitors[21]. Furthermore, RARα-deficient cells showed enhanced sensitivity to histone deacetylase inhibitors *in vitro* and *in vivo*[22]. These studies suggest that retinoid signal activation is necessary for histone deacetylase inhibitor activity. Hahn *et al*[23] used an HDAC inhibitor (valproicacid) as an enhancer to screen a small-molecule library for compounds inducing NB maturation, the top hit identified in the screen was all-trans-retinoic acid. These studies demonstrated that investigation of HDAC inhibitors and retinoids in combination are warranted to improve the anticancer activities in cancer.

Combination therapies improve the anticancer activities of retinoids in NB

Synergistic anti-tumour activity between histone deacetylase inhibitors and retinoids has been observed in a variety of preclinical models[24,25]. A study suggested that the HDAC inhibitor LAQ824 has a greater antitumor activity in combination with 13-*cis*-retinoic acid in melanoma tumors[24]. Another study showed that the intracranial tumors in ND2:SmoA1 mice treated with retinoid acid + SAHA + cisplatin showed a 4-fold increase in apoptosis over controls, and a 2-fold increase over animals receiving only SAHA or retinoid acid + SAHA[25]. We and others have shown that retinoids combined with histone deacetylase inhibitors are synergistic[26,27]. However, SAHA combined with 13-*cis*-retinoic acid, was well-tolerated in a phase Ⅰ/Ⅱ paediatric trial, but the best response for relapsed solid tumour patients was stable disease[28]. Recently, our study showed that 4-HPR+SAHA as a more effective therapy for NB than 13-*cis*-RA alone or with SAHA[29]. The 4-HPR + SAHA combination induced caspase-dependent apoptosis through activation of caspase 3, reduced colony formation and cell migration *in vitro*, and tumorigenicity *in vivo*. The 4-HPR and SAHA combination significantly increased mRNA expression of thymosin-beta-4 (Tβ4) and decreased mRNA expression of RARα. Importantly, the up-regulation of Tβ4 and down-regulation of RARα were both necessary for the 4-HPR + SAHA cytotoxic effect on NB cells. Moreover, Tβ4 knockdown in NB cells increased cell migration and blocked the effect of 4-HPR + SAHA on cell migration and focal adhesion formation[29]. This study demonstrates that Tβ4 is a novel therapeutic target in NB, and that 4-HPR and SAHA is a potential combination therapy for the disease.

CONCLUSION

A therapeutic role for retinoids and HDAC inhibitors in several human cancer types, including NB, is well established. However, retinoids and HDAC inhibitors are not completely effective anti-cancer agents when used alone; thus, a better understanding of their mechanism of actions will lead to more evidence-based retinoid combination therapies. Because differentiation is aberrant in NB, compounds that modulate transcription and induce differentiation, such as HDAC inhibitors and retinoids, are of particular interest. Further studies to understand the mechanism of drug actions and the clinical trials with large cohort of patients to determine the efficacy of HDAC inhibitors and retinoids for patients with high-risk NB are warranted.

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