An enzyme-linked immunosorbent assay to screen for inhibitors of the oncogenic anaplastic lymphoma kinase

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Abstract
The discovery of novel anti-cancer drugs targeting anaplastic lymphoma kinase (ALK), an oncogenic tyrosine kinase, raises the need for in vitro assays suitable for screening compounds for ALK inhibition. To this aim we have developed and optimized an ALK-specific enzyme-linked immunosorbent assay that employs a novel ALK peptide substrate and purified ALK kinase domain.

Reference

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The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase normally expressed in the developing nervous system. However, chromosomal translocations involving the ALK gene (2p23) lead to the expression of constitutively activated ALK fusion proteins such as NPM/ALK, in tissues outside the nervous system. ALK fusion proteins stimulate mitogenic and anti-apoptotic signaling pathways, leading to malignant transformation in cancers such as anaplastic large cell lymphoma (ALCL). Therefore, ALK represents a valid target for pharmaceutical intervention. In the present study, we describe the development of an enzyme-linked immunosorbent assay (ELISA) that can be used to rapidly screen compounds for their ability to inhibit ALK in vitro.

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Letters to the Editor

Malignant Lymphomas

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The discovery of novel anti-cancer drugs targeting anaplastic lymphoma kinase (ALK), an oncogenic tyrosine kinase, raises the need for in vitro assays suitable for screening compounds for ALK inhibition. To this aim we have developed and optimized an ALK-specific enzyme-linked immunosorbent assay that employs a novel ALK peptide substrate and purified ALK kinase domain.

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The anaplastic lymphoma kinase (ALK) is a receptor tyrosine kinase normally expressed in the developing nervous system. However, chromosomal translocations involving the ALK gene (2p23) lead to the expression of constitutively activated ALK fusion proteins such as NPM/ALK, in tissues outside the nervous system. ALK fusion proteins stimulate mitogenic and anti-apoptotic sig-
Figure 1. A. Production and purification of recombinant (6x)His-tagged ALK (rALK). rALK containing amino acids 1073–1459 (NCBI Accession Code: Q9UM73) was expressed in Sf9 cells using the MaxBac®2.0 baculovirus expression system (Invitrogen). rALK was purified on a Q-sepharose Fast Flow column followed by a HiTrap™-nickel column (Amersham-Pharmacia Biotech). Q-sepharose (lane 1) and HiTrap fractions (lane 2) were assessed for: (i) purity by SDS-PAGE and silver staining; (ii) the presence of ALK by anti-ALK1 immunoblotting; (iii) autophosphorylation activity by a radioactive kinase assay. B. Identification of a peptide substrate of rALK. Phosphorylation of the ARDIYRASFFRKGGCAMLPVK peptide#1 (200 µM) and the random polymer polyGlu4Tyr (0.1 mg/mL) by rALK was measured by an Ultrospec plate reader (Amersham-Pharmacia Biotech). Results are expressed as mean dpm ± s.dev. (n=3). IC50 values were determined using GraphPad prism software fitting data using nonlinear regression. C. Inhibition of NPM/ALK autophosphorylation by staurosporine. BaF3 pro-B murine cells transfected with NPM/ALK were treated for 2h with staurosporine. NPM/ALK tyrosine phosphorylation was determined by anti-phosphotyrosine (anti-pTyr) immunoblotting and protein loading was controlled by anti-ALK1 immunoblotting. Band density was determined by densitometry and expressed as a percent of the control.

Figure 2. The effect of staurosporine on ALK activity. Staurosporine concentration-response curves showing inhibition of peptide#1 phosphorylation by purified rALK determined in the radioactive assay (A) and the ALK-ELISA (B). A. The kinase reaction was performed as described in Figure 1B in the presence of staurosporine, for 10 mins. B. The ALK-ELISA was performed as described in Figure 1C in the presence of 30 or 300 µM ATP and staurosporine. Results are normalized to vehicle control and expressed as the mean ± s.dev. (n=3). IC50 values were determined using GraphPad prism software fitting data using nonlinear regression. C. Inhibition of NPM/ALK autophosphorylation by staurosporine. BaF3 pro-B murine cells transfected with NPM/ALK were treated with staurosporine for 2h, and cell lysates were analyzed for NPM/ALK tyrosine phosphorylation by immunoblotting using anti-ALK1 and antiphosphotyrosine antibodies. Band density was determined by densitometry and expressed as a percent of the control.
on ALK with the most potent derivatives, adaphostin and NSE 689857, having IC₅₀-values of 23 and 10 µM, respectively (data not shown). UCN-01, a 7-hydroxy stauroporine derivative, reported to have induced disease stability in a patient with ALK-positive ALCI in a phase I clinical trial, was also tested. UCN-01 inhibited ALK in the presence of 30 µM ATP (IC₅₀=5 µM), however no inhibition was observed at 300 µM ATP (data not shown). The low potency of UCN-01 and its lack of specificity for NPM/ALK transformed cells in proliferation assays (data not shown) suggest that UCN-01 does not target ALK. Therefore, potent and specific ALK inhibitors still need to be developed. The ALK-ELISA is a robust and accurate method suitable for middle and high-throughput screening that can be applied to the discovery of ALK inhibitors.

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Key words: ALCL, ALK, kinase assay, inhibitor screening.

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References


Malignant Lymphomas

Highly active antiretroviral therapy and outcome of AIDS-related Burkitt's lymphoma or leukemia. Results of the PETHEMA-LAL3/97 study

Short, intensive cycles of chemotherapy have resulted in improved survival in Burkitt's lymphoma/leukemia (BL) in adults. The prognosis of patients with immunodeficiency virus (HIV)-associated BL is considered to be poor, but these patients have seldom been treated with BL-specific protocols. However, a study (PETHEMA-LAL3/97) in which patients with BL were treated regardless of their HIV status failed to find differences between HIV-infected and immunocompetent individuals. Furthermore, patients who received highly active antiretroviral therapy (HAART) seemed to have a slightly better disease-free survival than those who did not (p=0.051). We extended the follow-up analysis to elucidate the role of HAART in the survival of HIV-infected patients included in the PETHEMA-LAL3/97 protocol.

Letters to the Editor

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We present the longer-term results of the multicenter PETHEMA LAL3/97 study on Burkitt’s lymphoma/leukemia carried out by the Spanish PETHEMA group. The diagnostic criteria, characteristics of treatment, response criteria and follow-up procedures were reported in the original analysis. Briefly, patients over 15 years old with newly diagnosed advanced BL (leukemic disease, lymphoma in stages III-IV or in stage II with a bulky mass) were treated, irrespectively of HIV status, with eight cycles of chemotherapy including alternating combinations of cytarabine, methotrexate, cyclophosphamide, ifosfamide, doxorubicin, teniposide, vincristine and dexamethasone. Triple drug HAART, including at least one protease inhibitor and two nucleoside reverse transcriptase inhibitors, was recommended from diagnosis for HIV-positive patients if they were not receiving it already and was continued thereafter. The present analysis includes an extended follow-up of the 14 original HIV-positive patients reported and 5 additional patients included in the protocol but previously excluded from analysis because of insufficient follow-up at that time. Overall survival (OS) and disease-free survival (DFS) were censored in July 2004 or date of last contact. Virological response to HAART was defined as having...