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Mutation Research 570 (2005) 9–15
Fundamental and Molecular Mechanisms of Mutagenesis
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Truncated ALK derived from chromosomal translocation t(2;5)(p23;q35) binds to the SH3 domain of p85-PI3K

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Received 2 August 2004; received in revised form 13 September 2004; accepted 14 September 2004
Available online 8 December 2004

Abstract

The chromosomal translocation t(2;5)(p23;q35) is associated with "Anaplastic large cell lymphomas" (ALCL), a Non Hodgkin Lymphoma occurring in childhood. The fusion of the tyrosine kinase gene—ALK (anaplastic lymphoma kinase) on chromosome 2p23 to the NPM (nucleophosmin/B23) gene on chromosome 5q35 results in a 80 kDa chimeric protein, which activates the "survival" kinase PI3K. However, the binding mechanism between truncated ALK and PI3K is poorly understood. Therefore, we attempted to elucidate the molecular interaction between ALK and the regulatory p85 subunit of PI3K. Here we provide evidence that the truncated ALK homodimer binds to the SH3 domain of p85.

This finding may be useful for the development of a new target-specific intervention.

Keywords: NPM/ALK; Interaction; p85-PI3K; SH2; SH3

1. Introduction

Anaplastic large cell lymphoma (ALCL) is a subgroup of non-Hodgkin's lymphomas (NHL) and accounts for about 30% of pediatric large cell lymphomas [1]. The majority has a characteristic chromosomal translocation t(2;5)(p23;q35) [2,3]. The rearrangement fuses the catalytic domain of the tyrosine kinase gene

Abbreviations: PI3K, phosphatidyl inositol 3′OH-kinase; SH2; SH3, src-homology 2; 3; S_{473}, serine 473; TetR, bacterial tetracycline repressor—it contains a dimerization motive which has no mammalian homologue; T_{308}, threonine 308; Y_{418}, tyrosine 418

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doi:10.1016/j.mrfmmm.2004.09.011

ALK (anaplastic lymphoma kinase) on chromosome 2p23 to the NPM (nucleophosmin/B23) gene on chromosome 5q35 [4] resulting in a chimeric protein of 80 kD [5]. The full-length form of ALK is abundantly expressed in the testis and the small intestine [4]. Murine ALK, which is 85% identical to human ALK at the amino acid sequence level [6], was shown to be restricted to the central and peripheral nervous system. Normal hematopoietic cells do not express ALK, and ALK expression in ALCL is confined to T-and Null-type lymphoid cells and is only rarely found in B-cell derived malignancies.

We have shown that normal primary rat embryo cells (REC) become immortalized by NPM/ALK. Homodimerization of ALK and its cytoplasmic localization are necessary for efficient immortalization of REC and for the cooperation of c-H-ras to transform normal REC into aggressively growing tumorigenic cell lines. This could be demonstrated with the derivative TetR/ALK [7].

Immortalization overrides G1 cell cycle arrest mechanisms and blocks concurring apoptotic mechanisms by activating cellular survival signaling pathways. NPM/ALK was shown to confer a mitogenic signal to PLC-γ when tyrosine Y_{664} is phosphorylated [8]. In addition another signaling pathway of NPM/ALK seems to engage PI3K [9,10]. PI3K phosphorylates the 3′-OH position of the inositol ring in inositol phospholipids thereby generating phosphatidyl inositols (PIs) PtdIns 3*P*, PtdIns (3,4)P_2, and PtdIns (3,4,5)P_3 [11,12]. The PIs bind with high affinity to the pleckstrin homology domains of PDK1 and of Akt [11,12], and facilitate the recruitment of these proteins to the cell membrane [13]. In this pre-activated state, PDK1 autophosphorylates at S_{241} (within its activation loop) resulting in its full activation, which is required for subsequent Akt-T_{308} phosphorylation and activation [14].

Former investigations suggested that phospho-Y_{418} (p-Y_{418}) of NPM/ALK binds to a SH2 domain of the regulatory p85 subunit of PI3K, thereby increasing its activity [10]. This was shown by competing for the NPM/ALK—p85 interaction with a oligo-peptide spanning the p-Y_{418} region of NPM/ALK. Site directed mutation of Y_{418} to phenylalanine (F) mimics a site that cannot become phosphorylated [8], and, therefore cannot interact with a SH2 domain. Surprisingly, a NPM/ALK-Y_{418}F mutant still bound to SH2-p85 in cell-free extracts and maintained PI3K activity in cell culture [10]. This apparent discrepancy raises the possibility that the ALK-p85 -interaction is not achieved by the binding of p-Y_{418} to the SH2-p85 domain but by another type of recruitment mechanism. Interestingly, the region adjacent to NPM/ALK-Y_{418}, is proline-rich thereby forming a basic protein sequence, which might represent a docking site for a SH3 adapter domain. At the N-terminus p85 contains such a SH3 domain. Therefore, we constructed SH3- and SH2 deletion mutants of p85 to test the type of interaction with ALK in intact cells. We used the NPM/ALK derivative TetR/ALK for our studies, because it homodimerizes and resides strictly cytoplasmic thereby avoiding biases through NPM-NPM/ALK heteromerizations and nuclear localization.

2. Experimental

2.1. Materials

Bovine p85 wt-cDNA was a kind gift from Julian Downward (Imperial Cancer Research Fund, London). Antibodies anti p-Akt (Thr 308; Cell Signaling #9275) 1:1000, p-Akt (Ser 473; Cell Signaling #9271) 1:1000, Akt (Cell Signaling #9272) 1:1000, p-Tyrosine (Zymed #61-5800) 1:1000, p85 (BD Biosciences #556399), 1:1000, ErbB2 (St. Cruz #sc-284) 1:1000, ALK (Zymed Laboratories #51–3900) 1:500, V5 (Invitrogen #R-960–25), 1:1000 were used for western blotting. Genistein (Upstate Biotechnology, Lake Placid NY) and sodium ortho-vanadate (Sigma #s-6508) were dissolved in DMSO (Sigma #D-8779).

2.2. Cells

Karpas 299 cells (DSMZ #ACC31) were cultured in RPMI (GIBCO) containing 10% FCS (GIBCO, Paisley, UK), 1% penicillin–streptomycin (GIBCO–BRL) and 1% Glutamax (GIBCO, Invitrogen).

Human kidney 293 cells were grown in DMEM (GIBCO) supplemented with 10% FCS and 1% penicillin–streptomycin. Cells were incubated at 37° in a humidified atmosphere containing 5% CO_2.

Primary human T-lymphocytes were isolated from peripheral blood of a healthy donor using the Ficoll density gradient–centrifugation method (Ficoll-Paque