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Review

Emerging importance of ALK in neuroblastoma

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ABSTRACT

Since the original descriptions of gain-of function mutations in anaplastic lymphoma kinase (ALK), interest in the role of this receptor tyrosine kinase in neuroblastoma development and as a potential therapeutic target has escalated. As a group, the activating point mutations in full-length ALK, found in approximately 8% of all neuroblastoma tumors, are distributed evenly across different clinical stages. However, the most frequent somatic mutation, F1174L, is associated with amplification of the MYCN oncogene. This combination of features appears to confer a worse prognosis than MYCN amplification alone, suggesting a cooperative effect on neuroblastoma formation by these two proteins. Indeed, F1174L has shown more potent transforming activity in vivo than the second most common activating mutation, R1275Q, and is responsible for innate and acquired resistance to crizotinib, a clinically relevant ALK inhibitor that will soon be commercially available. These advances cast ALK as a *bona fide* oncoprotein in neuroblastoma and emphasize the need to understand ALK-mediated signaling in this tumor. This review addresses many of the current issues surrounding the role of ALK in normal development and neuroblastoma pathogenesis, and discusses the prospects for clinically effective targeted treatments based on ALK inhibition.

1. Introduction

Identification of the anaplastic lymphoma kinase, ALK, as a therapeutic target in primary neuroblastoma represents a major research advance in this childhood cancer. The original discovery of ALK in 1994 resulted directly from investigations into the frequently observed t(2;5)(p23;q35) chromosomal rearrangement in anaplastic large cell lymphoma (ALCL), which fuses the cytoplasmic domain of ALK to the N-terminal portion of the nucleolar phosphoprotein, NPM [1]. Many other examples of aberrant ALK activation due to chromosomal rearrangement have been described in human cancers, including non-small-cell lung carcinomas (NSCLC), inflammatory myofibroblastic tumors (IMTs), diffuse large cell B-cell lymphomas and esophageal squamous cell carcinomas [2]. More recently, activating mutations of ALK were discovered in both familial and sporadic cases of neuroblastoma, an often fatal tumor that arises from neural crest cells of the noradrenergic lineage [3–6]. These findings are important because mutations in this receptor tyrosine kinase have displayed transforming potential in vivo [3] and their knockdown in neuroblastoma cells inhibits proliferation [3–6]. Thus, continued study of wild-type and mutated ALK holds considerable promise for deciphering the signaling networks that underlie neuroblastoma pathogenesis and for developing effective targeted therapies.

2. The ALK receptor tyrosine kinase

2.1. Structure

ALK has been classified as a member of the insulin receptor (IR) superfamily of receptor tyrosine kinases (RTKs) due to its high homology with other members of this family, such as the leukocyte tyrosine kinase (LTK), the insulin-like growth factor-1 receptor kinase (IGF1RK) and the insulin receptor kinase (IRK) [7]. ALK is located in humans at chromosome 2p23 and on the distal end of mouse chromosome 17 [8]. It is a single-chain 1620 amino acid (aa) transmembrane protein consisting of a 1030-aa extracellular domain, a 28-aa transmembrane-spanning domain and a 276-aa intracellular tyrosine kinase domain [9] (Fig. 1). The 177-kDa polypeptide encoded by the human ALK gene undergoes post-translational modifications, such as N-glycosylation, to generate a mature protein doublet of 220 and 190 kDa [10–12]. ALK migrates as two protein isoforms: the 220-kDa full-length receptor and the truncated 140 kDa protein that results from extracellular cleavage [13]. The functional relevance of this phenomenon is not

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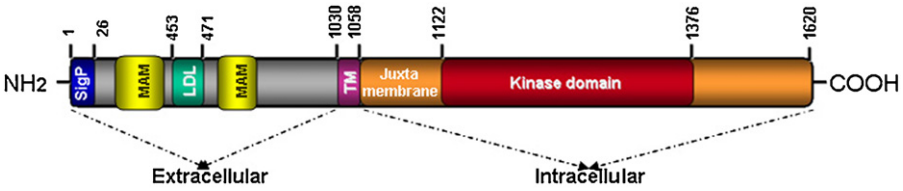

Fig. 1. Domain structure of ALK. The N-terminal, extracellular region of human ALK contains a signal peptide (aa 1–26), two MAM domains (aa 264–427 and 480–626), one LDL domain (aa 453–471) and a glycine-rich domain (aa 816–940). The transmembrane domain (TM) (aa 1030–1058) connects the extracellular and intracellular domains. The intracellular (cytoplasmic) domain contains the juxtamembrane (1058–1122) and the tyrosine kinase catalytic domains (aa 1122–1376) (SigP, signal peptide; TM, transmembrane), not drawn to scale.

clear, although the cleavage can be inhibited by an as yet unidentified factor secreted by Schwann cells [13].

The extracellular region of ALK comprises a 26-aa N-terminal signal peptide sequence, two MAM (meprin, A-5 protein, receptor protein tyrosine phosphatase mu) domains (aa 264–427 and 480–626) that are thought to have adhesive functions, one LDLa domain that forms a binding site for LDL and calcium, and a glycine-rich region [9,14]. This extracellular segment also contains the binding site for ligand binding [15–17] between aa 391 and 401 [2,15]. Both the extra- and intracellular domains are connected by a 28 aa transmembrane domain, which is followed by a 64-aa juxtamembrane domain. The tyrosine kinase catalytic domain (aa 1122–1376) contains a three-tyrosine kinase motif YXXXYY represented by Tyr1278, Tyr1282 and Tyr1283 within its activation loop (A-loop); these are major sites common to kinases in the insulin receptor family whose phosphorylation regulates kinase catalytic activity. Ligand binding leads to receptor dimerization and activation via *trans*-autophosphorylation of these tyrosine residues [7]. In addition, other phosphorylation sites have been described in the juxtamembrane domain (aa 1093–1096) and within the cytoplasmic domain (aa 1504–1507) that serve as binding sites for IR substrate-1 and Src homology 2 domain containing (SHC) proteins involved in downstream signaling [7].

2.2. Activation of ALK

Because of the high overall sequence identity within the A-loop regions of ALK and IGF1RK/IRK, it was thought that they shared common regulatory and activation mechanisms. IGF1RK/IRK maintain their inactive states by a number of autoinhibitory mechanisms including positioning the unphosphorylated A-loop so that it prevents the access of ATP and protein substrates to the ATP-binding pocket [18,19]. Kinase activation is regulated by the sequential phosphorylation of three major autophosphorylation sites, with the second tyrosine preferentially phosphorylated first, followed by the first tyrosine residue and lastly the third residue. In its inactive form, the A-loop occludes the ATP-binding pocket through the proximal A-loop DFG (Asp-Phe-Gly) motif ("DFG-out" conformation) and through pseudo-substrate binding of the second A-loop tyrosine residue to the phosphoacceptor site. When these negative regulatory restraints are overcome, the A-loop undergoes a conformational change and swings outward and away from the ATP-binding pocket, allowing unimpeded access of ATP ('DFG-in') and peptide substrate, leading to activation. However the autoinhibitory mechanisms in ALK appear to be different from those of IGF1RK/IRK, with ALK lacking many of the negative regulatory features of the inactive IGF1RK/IRK molecule. Firstly, preferential phosphorylation of the *first* tyrosine residue, Tyr 1278, occurs in ALK, this residue being critical for autoactivation of the ALK kinase domain and transforming activity [20]. In fact, ALK has minimal requirement for phosphorylation of the second and third tyrosine residues [21,22]. Secondly, ALK possesses narrower peptide substrate specificity when compared with the IGF1RK/IRK. These differences between ALK and IGF1RK/IRK were explained by two independent groups who recently reported the X-ray crystal structure of the ALK catalytic domain in its inactive conformation [21,22]. The A-loop of ALK has a unique autoinhibitory conformation in which a short helix at the proximal A-loop restricts the mobility of the N-terminal lobe, while the distal A-loop sterically obstructs a portion of the predicted peptide-binding region. This autoinhibitory mechanism of ALK that relies on intramolecular interactions between the N-terminal β-sheet and the DFG helix prevents binding of the peptide substrate and sequesters the first tyrosine residue, Tyr 1278, so that it is inaccessible for phosphorylation [22]. A single amino acid difference in the phosphoacceptor site as well as A-loop sequence differences account for the different sequence of tyrosine phosphorylation and the unique peptide substrate specificity seen in ALK compared to the IRKs [21]. Altogether, it appears that autoinhibition of the ALK tyrosine kinase domain is achieved by mechanisms similar to that used by EGFR rather than members of the insulin receptor family.

2.3. Normal expression

ALK is preferentially expressed in the central and peripheral nervous systems [7,10]. In mice, *in situ* hybridization studies showed that ALK mRNA expression is restricted to regions in the developing brain and peripheral nervous system (thalamus, hypothalamus, midbrain, cranial ganglia, and olfactory bulb as well as the enteric nervous system and dorsal root ganglia) during embryogenesis. Levels of ALK mRNA decrease after gestation, and ALK protein levels decline postnatally, remaining at low levels in the adult animal [10,23]. These findings are supported by immunohistochemical studies demonstrating consistently low levels of ALK in adult human CNS tissue samples [12], restricted to rare scattered neural cells, endothelial cells and pericytes in the brain [10,12,23].

2.4. Normal function

The normal function of the full-length ALK receptor is not entirely clear, although its predominant expression in the brain during development indicates that it likely plays an important role in the development and function of the nervous system [10,13,24]. Mice homozygous for a deletion of the ALK kinase domain develop normally, with no obvious anatomical abnormalities and a normal life-span [7]. However, these mice do exhibit increased basal dopaminergic signaling within the prefrontal cortex and an age-dependent increase in basal hippocampal progenitor proliferation, with concomitant enhanced performance in novel object recognition/location tests [25]. In *Drosophila melanogaster*, the receptor is vital for the development of visceral gut musculature, as the absence of dALK leads to loss of mesodermal founder cells responsible for gut development [26,27].