Anaplastic lymphoma kinase in human cancer

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Abstract

The receptor tyrosine kinases (RTKs) play a critical role, controlling cell proliferation, survival, and differentiation of normal cells. Their pivotal function has been firmly established in the pathogenesis of many cancers as well. The anaplastic lymphoma kinase (ALK), a transmembrane RTK, originally identified in the nucleophosmin (NPM)–ALK chimera of anaplastic large cell lymphoma, has emerged as a novel tumorigenic player in several human cancers. In this review, we describe the expression of the ALK–RTK, its related fusion proteins, and their molecular mechanisms of activation. Novel tailored strategies are briefly illustrated for the treatment of ALK-positive neoplasms.

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Introduction

Since the seminal description of the nucleophosmin (NPM)–anaplastic lymphoma kinase (ALK) fusion protein in anaplastic large cell lymphoma (ALCL; Morris et al. 1994, Shiota et al. 1994), many ALK chimeras have been described in inflammatory myofibroblastic tumors (IMTs; Griffin et al. 1999), diffuse large B-cell lymphoma (DLBCL; Arber et al. 1996), and more recently, in several epithelial neoplasms, including non-small cell lung cancer (NSCLC; Rikova et al. 2007, Soda et al. 2007), esophageal squamous cell carcinoma (SCC; Jazii et al. 2006, Du et al. 2007), colon (Lin et al. 2009), and breast carcinoma (Lin et al. 2009). ALK receptor expression, originally documented in a variety of cancer lines, has been documented in many neuronal tumors (Lamant et al. 2000, Miyake et al. 2002, 2005, Stoica et al. 2002, Osajima-Hakomori et al. 2005), glioblastoma (Powers et al. 2002, Shao et al. 2002, Grzelinski et al. 2005, Lu et al. 2005), and mesenchymal neoplasms including melanoma (Dirks et al. 2002) and rhabdomyosarcoma (Morris et al. 1994, 1997, Pulford et al. 1997, Falini et al. 1998, Cesnà et al. 2002, Pillay et al. 2002, Li et al. 2004). In this context, ALK overexpression or gain of function mutations have been demonstrated to be tumorigenic.

ALK expression in hematological disorders

ALCL, first described in 1985 (Stein et al. 1985), nowadays corresponds to a specific subtype of systemic peripheral T-cell lymphoma (Swerdlow et al. 2008). Most ALCL display chromosomal translocations of the ALK gene, although a subset, lacking these aberrations, is now recognized as a provisional entity (Swerdlow et al. 2008). ALK encodes a 210 kDa tyrosine kinase (TK) receptor (CD247) belonging to the insulin growth factor receptor super family. It is expressed at high levels in the nervous system during embryogenesis but only focally in the adult brain (Iwahara et al. 1997). Its presence outside of the nervous system is believed to be negligible in normal tissues. Although the physiologic role of ALK receptor in mammals is unknown, it might
be involved in neuronal differentiation, as suggested by its ability to induce neurite outgrowth in vitro (Souttou et al. 2001) and by its role in synapse formation in Caenorhabditis elegans and Drosophila melanogaster (Liao et al. 2004, Bazigou et al. 2007, Reiner et al. 2008).

Remarkably, Allouche (2007) has recently demonstrated that ALK (CD246) is a novel dependence receptor. Indeed, the ALK receptor is inactive in the absence of engaging ligand(s) and its expression results in enhanced apoptosis, whereas ALK activation, via a ligand-mediated engagement or as result of ALK fusion proteins, decreases apoptosis (Mourali et al. 2006).

Virtually, all ALK chimeras derive from genomic breakpoints, almost invariably located within the intron between the exons 19 and 20 (NM_004304.3), leading to the fusion of the intracytoplasmic domain of ALK (exons 20–29) with different partners, which provide dimerization domains (Chiarle et al. 2009, Fornari et al. 2009).

Many ALK-positive (ALK+) ALCL express the NPM–ALK fusion protein, derived from the t(2;5)(p23;q25) translocation (Jaffe et al. 2001). NPM1 is a multifunctional protein, which acts as a molecular chaperone in the transport of pre-ribosomal particles from the nucleus to the cytoplasm, although it plays a critical role in DNA repair, transcription, and genomic stability as well (Okuwaki 2008). The N-terminus domain of NPM1, within the ALK chimera, provides a dimerization domain, essential for chimera autophosphorylation, allowing the constitutive activation of the kinase and the firing of downstream signaling (Fujimoto et al. 1996, Bischof et al. 1997, Chiarle et al. 2008).

The oncogenic potential of ALK chimeras was first demonstrated in vivo in mice undergoing bone marrow transplantation with cells transduced with NPM–ALK construct (Kuefer et al. 1997). Similar results were obtained testing the transforming potential of fibroblasts containing NPM–ALK in vitro (Bai et al. 1998). In 2003, a mouse model was generated in which the expression of NPM–ALK, under the control of the CD4 promoter (Chiarle et al. 2003), showed the spontaneous development of T-cell lymphomas and/or plasmacytomas, confirming the lymphomagenic role of NPM–ALK, providing a valuable tool for the study of ALCL. These findings were then confirmed using additional mouse models (Turner & Alexander 2005).

Mutagenesis and functional studies have identified several NPM–ALK interacting molecules such as PLC-γ, IRS1, HSP90, GRB2, SHCC, JAK2/JAK3, PI3K, and STAT3/5 (Chiarle et al. 2008; Fig. 1).

**Phospholipase C-γ**

NPM–ALK controls cellular proliferation via the phospholipase C-γ (PLC-γ) docking in position Y664 of NPM–ALK. PLC-γ activation induces the hydrolysis of phosphatidylinositol (PIP2) into inositol triphosphate (IP3) and diacylglycerol (DAG), molecules that can modulate the release of Ca2+ from intracellular compartments and activate the serine/threonine protein kinase C (PKC). Ba/F3 cells (a pro-B line that requires IL3 for survival and growth) can grow in IL3-independent manner following the NPM–ALK transfection, while the use of the NPM–ALKY664F mutant completely disables their growth in the absence of IL3 (Bai et al. 1998).

**RAS**

ALK+ ALCL cell growth is largely dependent on the Ras–extracellular signal regulated kinase (ERK) pathway. ALK fusion proteins can engage the effectors IRS1, SHC, and GRB2 lead to the constitutive activation of Ras. Although IRS1 and SHC may not be required for transformation (Fujimoto et al. 1996), inhibition of ERK-1 and -2 leads to cell cycle arrest and block of proliferation.

Ras activation via mitogen-activated protein kinases (MAPK), ERK-1, and -2 regulates the phosphorylation of several transcription factors, including the AP-1 complex, which is believed to contribute to the ALCL neoplastic phenotype (i.e. CD30).

**Phosphatidylinositol 3 kinase**

NPM–ALK interacts directly and indirectly with PI3K (Bai et al. 2000). Following this association, the PI3K catalytic subunit (p110) leads to the activation of the PKB/AKT pathway. AKT, a serine/threonine kinase, is known to provide anti-apoptotic signals regulating several mediators, including caspase 9, BAD, NF-kB, and Fas ligand (Chiarle et al. 2008). Moreover, AKT, through the hyperphosphorylation of the transcription factor FOXO3a (Gu et al. 2004), increases cyclin D2 and inhibits p27 transcription, forcing G1 phase cell cycle arrest.

**c-Src**

c-Src is a TK receptor that plays a relevant role in cell migration, as well as in cell proliferation and growth. Its kinase activity is essential for the integrin-mediated adhesion and for morphological adaptation of cells. c-Src is normally maintained in a catalytically inactive conformation by molecular interactions via its SH2 and SH3 domains. pp60 (c-Src) is activated by NPM–ALK following its association with a tyrosine residue in position 418. Studies taking advantage of Src-specific inhibitors or RNA interference have shown that NPM–ALK-mediated activation of c-Src kinase is important for the growth of NPM–ALK-positive ALCL cells. SRC-family kinases may also contribute to the activation of VAV1, which was directly activated by...
NPM–ALK, leading to a sustained activation state of Cdc42 in ALCL cells (Ambrogio et al. 2008). Cdc42 regulates the shape and migration of ALCL cells and it is necessary for the growth and maintenance of lymphoma cells in vivo (Ambrogio et al. 2008).

Signal transducers and activators of transcription

Signal transducers and activators of transcription (STAT) proteins are a family of transcription factors first characterized for their role in cytokine signaling.
These proteins contain a site for specific tyrosine phosphorylation, which after modification results in a conformational rearrangement and dimerization through phosphotyrosine–SH2 domain interactions (Levy & Darnell 2002). Once STATs are phosphorylated, they dimerize and accumulate in the cell nucleus and bind to enhancer elements of target genes. Zamo et al. (2002) have first shown that STAT3 is the key effector molecule of the ALK-mediated signaling in ALCL and its activation is required for the maintenance of the neoplastic phenotype (Chiarle et al. 2005). NPM–ALK can directly phosphorylate STAT3 or can activate JAK3, which in turn can contribute to STAT3 activation (Chiarle et al. 2008). STAT3 phosphorylation results in an increased expression of BCL2, BCL-XL, survivin, and MCL-1 proteins, involved in anti-apoptotic processes. STAT3-mediated signal also leads to an uncontrolled proliferation, acting on cell cycle regulators such as cyclin D3 and c-myc (Amin et al. 2005), often overexpressed in ALK+ lymphoma (Chiarle et al. 2003). Cooperation between NPM–ALK and JAK/STAT pathway might also lead in certain context to the STAT5 activation (Nieborsowska-Skorska et al. 2001), although in T-cell, STAT3 acts as a STAT5 repressor (Zhang et al. 2007).

ALK fusion proteins

In addition to NPM–ALK, many other fusion proteins can be expressed in ALCL, namely ALK lymphoma oligomerization partner on chromosome 17 (ALO17; Cools et al. 2002), TRK-fused gene (TFG; Hernández et al. 1999, 2002), moesin (MSN; Tort et al. 2001), tropomyosin 3 and 4 (TPM3 and TPM4; Lamant et al. 1999, Siebert et al. 1999, Meech et al. 2001), 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC; Colleoni et al. 2000, Ma et al. 2000, Trinei et al. 2000), non-muscle myosin heavy chain (MYH9; Lamant et al. 2003), and clathrin heavy chain (CLTC–ALK; Touriol et al. 2000; Table 1).

Table 1  Chromosomal translocations involving anaplastic lymphoma kinase gene in cancers

<table>
<thead>
<tr>
<th>Disease</th>
<th>Fusion protein</th>
<th>Chromosomal abnormality</th>
<th>Principal references</th>
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<tbody>
<tr>
<td>ALCL</td>
<td>NPM–ALK</td>
<td>t(2;5)(p23;q35)</td>
<td>Morris et al. (1994) and Shiota et al. (1994)</td>
</tr>
<tr>
<td>ALCL</td>
<td>ALO17–ALK</td>
<td>t(2;17)(p23;q25)</td>
<td>Cools et al. (2002)</td>
</tr>
<tr>
<td>ALCL</td>
<td>TFG–ALK</td>
<td>t(2;3)(p23;q21)</td>
<td>Hernández et al. (1999, 2002)</td>
</tr>
<tr>
<td>ALCL</td>
<td>MSN–ALK</td>
<td>t(2;X)(p32;q11–12)</td>
<td>Tort et al. (2001, 2004)</td>
</tr>
<tr>
<td>ALCL</td>
<td>TPM3–ALK</td>
<td>t(1;2)(q25;p23)</td>
<td>Lamant et al. (1999) and Siebert et al. (1999)</td>
</tr>
<tr>
<td>ALCL</td>
<td>TPM4–ALK</td>
<td>t(2;19)(p23;p13)</td>
<td>Meech et al. (2001)</td>
</tr>
<tr>
<td>ALCL</td>
<td>ATIC–ALK</td>
<td>inv(2)(p23;q35)</td>
<td>Colleoni et al. (2000), Ma et al. (2000), and Trinei et al. (2000)</td>
</tr>
<tr>
<td>ALCL</td>
<td>MYH9–ALK</td>
<td>t(2;22)(p23;q11–2)</td>
<td>Lamant et al. (2003)</td>
</tr>
<tr>
<td>ALCL</td>
<td>CLTC–ALK</td>
<td>t(2;17)(p23;q23)</td>
<td>Touriol et al. (2000)</td>
</tr>
<tr>
<td>IMT</td>
<td>TPM3–ALK</td>
<td>t(1;2)(q25;p23)</td>
<td>Lawrence et al. (2000)</td>
</tr>
<tr>
<td>IMT</td>
<td>TPM4–ALK</td>
<td>t(1;19)(p23;p13)</td>
<td>Lawrence et al. (2000)</td>
</tr>
<tr>
<td>IMT</td>
<td>CLTC–ALK</td>
<td>t(2;17)(p23;q23)</td>
<td>Bridge et al. (2001) and Patel et al. (2007)</td>
</tr>
<tr>
<td>IMT</td>
<td>SEC31L1–ALK</td>
<td>t(2;4)(p23;q21)</td>
<td>Panagopoulos et al. (2006)</td>
</tr>
<tr>
<td>IMT</td>
<td>RANBP2–ALK</td>
<td>t(2;2)(p23;q13)</td>
<td>Ma et al. (2003)</td>
</tr>
<tr>
<td>IMT</td>
<td>CAR–ALK</td>
<td>t(1;11)(p23;p15;q31)</td>
<td>Cools et al. (2002) and Debelenko et al. (2003)</td>
</tr>
<tr>
<td>NSCLC</td>
<td>EML4–ALK</td>
<td>inv(2)(p21t;p23)</td>
<td>Rikova et al. (2007) and Soda et al. (2007)</td>
</tr>
<tr>
<td>NSCLC</td>
<td>TFG–ALK</td>
<td>t(2;3)(p23;q21)</td>
<td>Rikova et al. (2007)</td>
</tr>
<tr>
<td>DLBCL</td>
<td>NPM–ALK</td>
<td>t(2;5)(p23;q35)</td>
<td>Adam et al. (2003) and Onciu et al. (2003)</td>
</tr>
<tr>
<td>DLBCL</td>
<td>CLTC–ALK</td>
<td>t(2;17)(p23;q23)</td>
<td>De Paepe et al. (2003)</td>
</tr>
<tr>
<td>DLBCL</td>
<td>Unknown</td>
<td>ins(3’ALK)(4q22–24)</td>
<td>Stachurski et al. (2007)</td>
</tr>
<tr>
<td>DLBCL</td>
<td>SQSTM1–ALK</td>
<td>t(2;5)(p23;1;q35–3)</td>
<td>Takeuchi et al. (2010)</td>
</tr>
<tr>
<td>DLBCL</td>
<td>SEC31A–ALK</td>
<td>ins(4)(2;4)(?;q21) t(2;4)(p24;q21)</td>
<td>Bedwell et al. (2010) and Van Roosbroeck et al. (2010)</td>
</tr>
<tr>
<td>SCC</td>
<td>TPM4–ALK</td>
<td>t(2;19)(p23;p13)</td>
<td>Du et al. (2007) and Jazii et al. (2006)</td>
</tr>
<tr>
<td>RCC</td>
<td>VCL–ALK</td>
<td>t(2;10)(p23;q22)</td>
<td>Debelenko et al. (2010)</td>
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role for the appropriate classification of ALCL, demonstrating that ~60–80% of all ALCL are ALK+ (Webb et al. 2009). It is important to underline that ALK− ALCL are indistinguishable from ALK+ ALCL using morphological criteria alone. Therefore, the expression of ALK has become a key factor, not only for a proper diagnosis, but also for the precise ALCL stratification, providing relevant prognostic and therapeutic information. Since ALCL share a distinct gene expression profile, it has been postulated a putative common origin and/or common transformation pathway(s) for all ALCL (Piva et al. 2010). A single ALK lesion, although essential for transformation, requires additional genetic defects, which are however yet to be determined. The actual impact of insect bites in the pathogenesis of ALK+ ALCL remains to be elucidated (Formari et al. 2009, Lamant et al. 2010).

Interestingly, as underlined in the fourth edition of the WHO classification (Swerdlow et al. 2008), both ALK+ and ALK− ALCL are characterized by frequent diffusion through sinuses and a cohesive growth pattern that can mimic metastatic carcinoma in the lymph node. They consist of very large lymphomatous tumors that in the ALK+ tumors usually acquire a kidney- or horseshoe-shaped normal profile that justifies the term ‘hallmark cells’. Besides the classical type, almost exclusively formed by large cells with a few reactive elements, ALK+ ALCL display some morphological variants: lympho-histiocytic, small cell, mixed, and Hodgkin-like cells. Under these circumstances, the expression of ALK by the neoplastic cells is of paramount importance for the distinction of the process from a hyperimmune reaction, PTCL-NOS, and nodular sclerosing Hodgkin lymphoma respectively. It is still a matter of debate whether similar variants are also observed in the setting of ALK− ALCL: possibly the lympho-histiocytic and Hodgkin-like ones do occur, although their recognition require negativity for PAX5/BSAP and occurrence of T-cell markers and possible clonal TCR rearrangements.

ALK+ ALCL most frequently occur in the first decades of life with a typical male preponderance, although ALK+ ALCL can also be seen in older individual at lower frequency; while ALK− ALCL arise most commonly in older patients (peak of incidence in the sixth decade) with a lower male preponderance (Shiota et al. 1995, Falini et al. 1999, Stein et al. 2000, Savage et al. 2008). ALK+ ALCL patients have longer disease-free survival and better overall survival (OS) than ALK− cases (5 year OS: 70–80 vs 33–49%) following CHOP-based chemotherapy (Brugieres et al. 1998, 2000, Falini et al. 1999, Stein et al. 2000, Williams et al. 2002, Savage et al. 2008), although these differences disappear if ALCL patients are stratified by stage (Savage et al. 2008).

Finally, it should be considered that the clinical outcome of ALCL is also influenced by the age of the patients, with a better survival in younger individuals. This may explain the more favorable clinical course of ALK+ ALCL most frequently occurring in children and young adults.

Notably, an aberrant ALK expression has been detected in a minute subset of B-NHL (Delsol et al. 1997, Adam et al. 2003, Chikatsu et al. 2003, De Paepe et al. 2003, Gascoyne et al. 2003, Onciu et al. 2003, Reichard et al. 2007). ALK+ DLBCL often carry the t(2;17) translocation (Clathrin/ALK), while NPM–ALK or SEC31A–ALK proteins are less frequently expressed (Van Roosbroeck et al. 2010). Histologically, they display monomorphic, large immunoblastic/plasmablastic cells, which are CD138+, EMA+, CD4+, and cytoplasmic IgA+ positive but lack CD30 and B-cell-restricted markers (Delsol et al. 1997, Reichard et al. 2007). ALK+ DLBCL are characterized by an aggressive outcome and poor response to treatment (Reichard et al. 2007, Stachurski et al. 2007, Choung et al. 2008, Lee et al. 2008, Momose et al. 2009).

Finally, Chan et al. (2008) have described three cases of systemic histiocytosis, presenting in early infancy, expressing ALK or the TPM3–ALK chimeras. It is unclear whether these disorders are indeed true malignancies or due to an aberrant hyperproliferation of macrophages and dendritic cells, driven by the ectopic ALK expression.

**ALK expression in non-hematological disorders**

**ALK in mesenchymal neoplasms**

The IMTs are benign lesions of mesenchymal origin, composed of spindle cells, mixed with plasma cells and lymphocytes (Gleason & Hornick 2008), originally thought to represent a reactive post-inflammatory condition rather than a neoplastic process (Umiker & Iverson 1954). In 1999, Griffin et al. reported the first ALK gene rearrangements in these disorders. Further studies have subsequently documented the presence of different ALK-fusion proteins, all sharing the ALK kinase domain, fused to different partners, eventually leading to TPM4–ALK (Lawrence et al. 2000), ATIC–ALK (Debiec-Rychter et al. 2003), CLTC–ALK (Bridge et al. 2001, Patel et al. 2007), CARS–ALK (Cools et al. 2002, Debelenko et al. 2003), RANBP2–ALK (Ma et al. 2003), and SEC31L1–ALK (Panagopoulos et al. 2006) fusion proteins. It is believed that 35–60% of all IMTs display ALK rearrangements, which more often are seen in lesions of young individuals (Lawrence et al. 2000, Coffin et al. 2001, Cook et al. 2001).
Among soft tissue tumors, Cessna et al. (2002) first reported two cases of rhabdomyosarcoma (RMS), with embryonal, alveolar features, and the NPM–ALK translocation. Subsequently, using an immunohistochemical approach, ALK expression was confirmed in 53% of alveolar RMS and 23% of embryonal or unclassifiable RMS, which can display ALK amplification (Corao et al. 2009).

**ALK and neural tumors**

Neuroblastoma is the most common extracranial solid tumor of childhood, derived from neural crest cells of the sympatho-adrenal lineage (Park et al. 2008). Although the clinical course of these patients is heterogeneous, many neuroblastomas are incurable, with poor long-term survival (Matthay et al. 1999), accounting for 15% of all pediatric oncology deaths (Maris et al. 2007).


Neuroblastoma-associated ALK–RTK mutations induce a constitutive activation of the receptor, which activates several downstream molecules (Osajima-Hakomori et al. 2005) imposing a transformed phenotype. Indeed, the genetic (Mosse et al. 2008) or pharmacological inhibition of ALK-mutated species (George et al. 2008, McDermott et al. 2008) results in a decreased tumor growth. On the other hand, the role of wt-ALK–RTK remains elusive, since its expression might be simply linked to lineage constrains and/or unique neuronal differentiation stage(s) (Dirks et al. 2002).

Powers et al. (2002) first demonstrated that some primary glioblastoma and established cell lines expressed wt-ALK–RTK as well as pleiotrophin (PNT), an ALK-putative ligand. Glioblastoma often displays deregulated RTKs signaling, which plays a key role in their development and tumor outgrowth (Nister et al. 1991, Nishikawa et al. 1994). Interestingly, the ribozyme-mediated targeting of ALK was shown to reduce tumor growth of glioblastoma xenografts and increase apoptosis. Finally, the ablation of both PNT and ALK strongly enhances their individual antiproliferative effects (Grzelinski et al. 2009).

**ALK in epithelial cancers**

In the last decade, it has also become evident that many types of non-lymphoid tumors display a deregulated activation of ALK. This was first suggested by the work of Dirks et al. (2002), who originally documented the presence of ALK mRNA in many cancer cell lines derived from thyroid, small cell lung, breast carcinoma, and many other tumors.


Finally, two different variants involving the KIF5B and ALK genes have been described in a small subset of NSCLC (Takeuchi et al. 2009, Wong et al. 2011).

Collectively, these studies have pointed out the presence of several shared features among ALK+ lung cancers: i) ALK fusions are mainly restricted to adenocarcinoma in patients with minimal or absent smoking story and young age of onset; ii) ALK rearrangements are mutually exclusive with other lung-associated genetic abnormalities such as EGFR and KRAS mutations; and iii) ALK translocations are not influenced by ethnic/racial differences, in contrast with EGFR mutations (Paez et al. 2004).

Notably, the univocal identification of ALK+ NSCLC patients remains quite problematic. Indeed, the recognition of ALK translocations by FISH can be technically demanding and sometimes questionable.
Similarly, the detection of ectopic ALK fusion proteins by immunohistochemistry is problematic as well (Inamura et al. 2008, Takeuchi et al. 2008, Martelli et al. 2009), and once FISH, immunohistochemistry, and RT-based approaches are combined, an overall consensus is reached in 80% of the cases (M Volante, personal communication, 24 November 2010). Moreover, normal lung epithelial and lymphoid cells can display ALK genetic lesions (Martelli et al. 2009, Sozzi et al. 2009).

ALK inhibitors, such as PF-2341066 or NPV-TAE-684 first in mouse models (Christensen et al. 2007, Galkin et al. 2007, Zou et al. 2007, McDermott et al. 2008, Soda et al. 2008) and more recently in clinical trials, have shown their therapeutic potential. Indeed, the data with crizotinib in a recent Phase II study have demonstrated an objective response rate of 57% and a disease control rate of 87% in NSCLC patients (Kwak et al. 2010). These findings are very impressive, although longer follow-up and different clinical trials may be required to conclusively assess the efficacy of a single drug regimen and its efficacy in naïve patients. Finally, the occurrence of ALK overriding resistance has to be precisely appraised and its molecular mechanism(s) dissected (Martinsson et al. 2010).

Perez-Pinera et al. (2007) first documented the ALK ectopic expression in a very large number of breast neoplasms, demonstrating detectable levels of ALK protein in normal breast epithelium and other non-epithelial elements by immunohistochemistry. Notably, the PNT knockdown in breast cancer cells can result in a decreased tumor growth in vitro (Fang et al. 1992, Garver et al. 1994, Riegel & Wellstein 1994) and in vivo (Zhang et al. 1997). These findings suggested a pathogenetic role of the wt-ALK–RTK in this disease. Supporting findings have been provided by Lin et al. (2009), who have documented the presence of EML4–ALK transcripts in ~2-5% of breast cancers and showed that ALK ablation leads to cell growth impairment. Analogous data have been generated in colon cancers (Lin et al. 2009) and very recently in renal cell carcinoma (Debelenko et al. 2010). The significance of ALK deregulation in breast and colon tumors remains unclear and its pathogenetic significance needs further confirmation (Fukuyoshi et al. 2008).

Finally, among epithelial cancers, squamous cell carcinoma (SCC) of the esophagus (SCCE) represents the sixth most common entity with the highest incidence rates in China, Iran, and developing countries. Deregulated ALK fusion proteins expression has been documented in SCCE, originally in Iranian patients by Jazìì et al. (2006) and subsequently confirmed in a cohort of Chinese individuals (Du et al. 2007).

In conclusion, the list of solid neoplasms positive for ALK is continuously growing (i.e. prostate cancer, etc. E Medico and G Inghirami, personal communication). These findings will definitively foster the execution of more frequent systematic molecular analyses and the development of reliable clinical diagnostic tests.

Innovative therapeutic approaches for ALK tumors

The ablation of ALK protein expression was originally obtained by ALK-specific small interfering RNA (siRNA) duplexes or selective ribozyme (Hubinger et al. 2003). These original studies showed that the ALK knockdown leads first to a cell cycle arrest, followed by massive apoptosis in vitro and/or in vivo (Piva et al. 2006). These original findings were first confirmed applying ALK-specific small molecules (Wan et al. 2006, Galkin et al. 2007) and more recently were supported by other novel ATP-competitive inhibitors (Li & Morris 2008, Cheng & Otte 2010). Since then, we have witnessed an increasing interest in this field, strongly encouraged by the discovery of a growing number of ALK+ cancers (Li & Morris 2008, Webb et al. 2009, Cheng & Otte 2010). As a result, the first ALK inhibitor, PF-2341066, an ATP competitor, targeting both c-Met and ALK (Christensen et al. 2007), has recently reached the clinical arena in the treatment of ALK+ NSCLC tumors, and other small molecules have just reached the clinics (LDK378) or are in pre-clinical stages (CEP28122, CEP37440, AP-26113, TAE-684, etc.). Meanwhile, several trials have also been opened for ALCL and neuroblastoma patients (http://www.ClinicalTrials.gov/). It is postulated that many compounds could soon reach the clinics (Webb et al. 2009, Cheng & Otte 2010).

Since ALK signaling activates multiple downstream molecules, i.e. PI3K/AKT, JAK/STAT3 and 5, mTOR, and SRC, it is reasonable to speculate that several small molecules, targeting key effectors within these pathways, will be investigated in ALK+ cancer patients. Considering the exquisite oncogenic addition of ALK+ ALCL to STAT3 (Piva et al. 2006), inhibition of this transcription factor could provide a novel therapeutic avenue. Nevertheless, because there is an enormous redundancy of signal transduction pathways in any given tumor, it is conceivable that we will be obliged to use disease/patient-specific cocktails to successfully knockdown multiple players among different pathways. This might be the case for those neoplasms displaying partial oncogenic addition to ALK and/or capable of executing counteracting resistant mechanisms. In this context, targeting EGFR, c-src, and MEK may also be considered. Finally, immunological strategies, in combination with conventional or small molecule approaches, could be considered to enhance anti-tumor responses or to gain the complete eradication of cancer cells.
Final remarks

Since the original discovery by Morris et al. (1994) of the first ALK translocation, we have witnessed pivotal discoveries that led to a deeper understanding of the mechanisms leading to ALK-mediated transformation and tumor maintenance of ALCL. Now, a similar knowledge is mandatory for all other ALK+ neoplasms. Dissecting this landscape is essential for the design of tailored therapies, for predicting therapeutic failures, and to overcome them. We hope that a dedicated effort will also be placed to fully understand the physiological role of the ALK receptor and to discover its ligand(s). Understanding the physiological role of ALK will be necessary for the development of clinical-grade diagnostic assays and for the design and implementation of immune-based therapeutic approaches.

Declaration of interest

The authors declare that there is no conflict of interest that could be perceived as prejudicing the impartiality of the research reported.

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