ALK biology in health and disease

Ruth H. Palmer

Background
Anaplastic lymphoma kinase (ALK) was initially discovered as a 2:5 chromosomal translocation in Anaplastic Large-Cell Non-Hodgkin’s lymphoma (ALCL), that produces the NPM-ALK fusion oncogene (1). The ALK receptor tyrosine kinase (RTK) is now a significant player in clinical oncology and has been implicated in many cancer types of cancer, arguably most well known in the form of the EML4-ALK oncogene in non-small cell lung cancer (NSCLC). The activation of ALK in tumors such as lung adenocarcinoma, has led to the FDA approval of ALK tyrosine kinase inhibitors (TKIs) such as crizotinib, ceritinib, and alectinib for use in a variety of ALK positive patient populations. Our knowledge of ALK function under normal conditions is more limited, but a number of studies in model organisms and cell models have increased our understanding significantly. Here we discuss the present state of knowledge regarding ALK biology, ranging from the model organisms C.elegans and Drosophila melanogaster, to human disease together with their potential implications.

Discussion
ALK as an oncogene
ALK activation in cancer arises not only as a result of the more familiar chromosomal translocation events such as EML4-ALK (2, 3) and NPM-ALK (1), but also by mutation of the full length ALK RTK (4). Over 30 different ALK translocations have been described in a wide variety of tumor types, including ALCL, NSCLC, IMT, DCLCL among others, leading to a complex picture of ALK fusion partners (Fig. 1). While the overall picture involves any players, some key general features of various ALK fusions can be stated based on studies to date:

- Dimerization of the ALK kinase domain via the fusion protein partner leading to trans-autophosphorylation and activation of ALK kinase domain;
- Expression of the ALK fusion protein in tissues reflecting the expression pattern of the partner proteins;
- Subcellular localization of the fusion protein that is influenced by the ALK partner protein.

The targets of the ALK fusion proteins are likely include both physiological and aberrant pathways, given that ALK activity has lost its normal spatial and temporal constraints, which are now determined by the respective ALK fusion partner (5). The ALK fusion proteins exhibit constitutively active kinase domains that are sensitive to inhibition by ATP-analogues that bind
within the active site of the kinase. There are now a substantial number of these inhibitors, a number of which are in various stages of clinical trial or FDA approved for clinical use (6). These inhibitors have clinical effect, albeit limited, due to the appearance of resistance (7–9), with an estimated 20 percent of resistance involving secondary activating point mutations in the ALK kinase domain that allow the mutated kinase domain to evade the inhibitory block.

Overexpression of ALK of has been reported in many types of cancer cell lines and human tumor samples (4). Less frequent is the mutation of the ALK RTK in the context of the full length receptor. One clear example is in neuroblastoma, where ALK can also be amplified and/or overexpressed. The strongest genetic evidence for ALK as an oncogenic driver comes from neuroblastoma, where approximately 80 percent of familial cases exhibit ALK mutation (10). To date in neuroblastoma greater than 20 different mutants have been described, although the vast majority fall within several hotspots residues within the kinase domain (F1174, R1275 and F1245) (4, 10–13). The majority of neuroblastoma mutations in ALK are single amino acid changes within the kinase domain which are either:

- Ligand-independent constitutively active ALK mutations;
- Ligand-dependent ALK mutants;
- Kinase inactive mutant versions of ALK.

As with the ALK fusion proteins, the ALK mutant RTKs described in neuroblastoma are sensitive to ALK kinase inhibitors. However, in common with ALK fusions harboring resistance mutations, the various ALK mutants from neuroblastoma exhibit differential responses to inhibition, dependent on the particular compound employed and its molecular interface. ALK function has been linked with Neurofibromatosis type 1 (Nf1) in the fruit fly, where dAlk functions an upstream activator of dNf1-regulated Ras signaling (14). Interestingly, like ALK, Nf1 is genetically implicated in neuroblastoma (15). These findings have led to the proposal of ALK as a potential therapeutic target in Neurofibromatosis type 1 as well as in neuroblastoma.

ALK ligands

ALK signaling has been well studied in model systems such as Drosophila melanogaster (Alk), Caenorhabditis elegans (SCD-2), and Danio rerio (DrLtk, DrAlk). In the invertebrate systems ALK activating ligands - Jelly-belly (Jeb) in flies (16, 17) and Hen-1 in C. elegans - have been identified (18). To date, no Jeb-like ligand has been reported to activate ALK signaling in vertebrate systems. However, a recent report identified long chain heparins as activating ALK ligand (19). While the picture is probably not complete, at the current time the activating ligands for ALK receptors in humans seem to differ substantially from those in invertebrate systems.

Mouse models addressing ALK function during development and disease

While the ALK signaling pathway plays a critical role in the development of the fruit fly (16, 17), this is not the case in mouse models that have been investigated. A number of articles have examined the effect of deletion of ALK in mice finding that loss of ALK does not result in
serious phenotypes, rather these mice exhibit mild behavioral and neural phenotypes (20, 21). A double knock-out, in which both ALK and the related LTK receptor were deleted, is also viable, suggesting that neither of these RTKs is critically required for development (20). The subtle phenotypes observed in the ALK knock-out mice are encouraging when considering treatment of young patients carrying ALK mutations with ALK TKIs, where published pediatric trials results indicate that crizotinib may be an effective therapeutic treatment strategy for some categories of children with ALK positive tumors (22).

In parallel with the progress being made in our understanding of the role of ALK signaling during developmental processes in the genetic model systems, mouse models have been

Fig. 1. Signaling via wild type and oncogenic ALK. Schematic general overview of signaling via human ALK. Activation of human wild type ALK by heparin binding to the extracellular domain of ALK has been reported to induce ALK dimerization and activation. Gain-of-function ALK mutations have been reported in neuroblastoma, while the ALK fusions oncogenes exhibit fusion partners that serve to dimerize and activate the tyrosine kinase activity of ALK. ALK mediates signalling via the Ras/MAPK, PI3-kinase/TOR/PKB/Akt, ERK5, PLC-γ, Rap1, JAK/STAT, Rac-CDC42 and the Jun pathways. Proteins such as SHH-GLI1, NIPA, Src, IRS1, p130-CAS, Shc, Grb2, C3G, c-Cbl, CrkL, and Frs-2 interact and are phosphorylated by ALK upon activation. ALK also regulates a number of genes at the transcriptional level, some which have been validated, such as MYCN, JunB, DEBPB, and BCL2A1.
developed to understand the role of ALK in cancer. Genetically modified mouse models exist for e.g. NPM-ALK and EML4-ALK fusions, while for neuroblastoma the collaborative relationship of ALK with the MYCN oncogene has been exploited in ALK neuroblastoma models (23–25).

Future Directions

Clearly the signaling pathways used by either different ALK fusion proteins or the full-length activated receptor have common components. However, given the variable cellular context downstream ALK signaling events can be expected to exhibit differences, based on tumor/tissue type and, where appropriate, the identity of the ALK fusion partner proteins. Future work to understand the key signaling factors in the various ALK positive tumor types will be important. We now have an impressive array of ALK kinase inhibitors at hand; important outstanding questions now include how to combine these most effectively with other approaches to avoid the appearance of resistance. Understanding the underlying ALK biology in each scenario is a significant challenge, but should provide information to improve our ability to inactivate the ALK signaling pathway. Here the role of ALK during development, in the neural crest and the CNS among other tissues will likely provide important information on ALK function in the future. Issues that are also important to address for future therapeutic treatments include the further exploration of heparin as an ALK ligand in mammalian systems, which has the potential to impact our understanding of ALK activation scenarios in particular where this RTK is overexpressed in cancer. In summary, a combination of model systems, from worms to mice, together with patient data analysis and a close clinical interaction will be key to our future understanding of ALK in both health and disease.

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