The pathobiology of the oncogenic tyrosine kinase NPM-ALK: a brief update

Raymond Lai and Robert J. Ingham

Ther Adv Hematol
(2013) 4(2) 119–131
DOI: 10.1177/2040620712471553

Abstract: Extensive research has been carried out in the past two decades to study the pathobiology of nucleophosmin-anaplastic lymphoma kinase (NPM-ALK), which is an oncogenic fusion protein found exclusively in a specific type of T-cell lymphoid malignancy, namely ALK-positive anaplastic large cell lymphoma. Results from these studies have provided highly useful insights into the mechanisms by which a constitutively tyrosine kinase, such as NPM-ALK, promotes tumorigenesis. Several previous publications have comprehensively summarized the advances in this field. In this review, we provide readers with a brief update on specific areas of NPM-ALK pathobiology. In the first part, the NPM-ALK/signal transducer and activator of transcription 3 (STAT3) signaling axis is discussed, with an emphasis on the existence of multiple biochemical defects that have been shown to amplify the oncogenic effects of this signaling axis. Specifically, findings regarding JAK3, SHP1 and the stimulatory effects of several cytokines including interleukin (IL)-9, IL-21 and IL-22 are summarized. New concepts stemming from recent observations regarding the functional interactions among the NPM-ALK/STAT3 axis, β catenin and glycogen synthase kinase 3β will be postulated. Lastly, new mechanisms by which the NPM-ALK/STAT3 axis promotes tumorigenesis, such as its modulations of Twist1, hypoxia-induced factor 1α, CD274, will be described. In the second part, we summarize recent data generated by mass spectrometry studies of NPM-ALK, and use MSH2 and heat shock proteins as examples to illustrate the use of mass spectrometry data in stimulating new research in this field. In the third part, the evolving field of microRNA in the context of NPM-ALK biology is discussed.

Keywords: NPM-ALK, STAT3, anaplastic large cell lymphoma, oncogenic tyrosine kinase, signalling

NPM-ALK and ALK-positive anaplastic large cell lymphoma

It has been 18 years since the identification and initial characterization of the fusion protein NPM-ALK, which is the result of a specific chromosomal translocation that brings the nucleophosmin (*NPM*) gene on chromosome 5q35 to the anaplastic large cell lymphoma kinase (*ALK*) gene on 2p23 [Morris *et al*. 1994; Shiota *et al.* 1994]. Since its discovery, the oncogenic role of NPM-ALK has been established using a variety of experimental models, and the mechanisms by which NPM-ALK exerts its oncogenic effects have been extensively investigated and summarized [Amin and Lai, 2007; Chiarle *et al.* 2008; Palmer *et al.* 2009; Inghirami and Pileri, 2011]. The role of *ALK* in several types of ALK-expressing solid tumors as well as the therapeutic use of ALK inhibitors in treating these cancers also have been reviewed in a number of recent papers [Azarova *et al.* 2011; Crystal and Shaw, 2011; Ogawa *et al.* 2011; Sasaki and Janne, 2011]. In this article, we intend to provide a brief update on the pathobiology of NPM-ALK in the context of ALK-positive anaplastic large cell lymphoma (ALK+ALCL). Our focus will be on a number of specific areas of NPM-ALK pathobiology our laboratories have recently been studying, as well as specific aspects of NPM-ALK signaling that have not been reviewed in detail elsewhere.

The discovery and the initial characterization of NPM-ALK have been detailed elsewhere [Amin and Lai, 2007; Chiarle *et al.* 2008; Palmer *et al.* 2009; Inghirami and Pileri, 2011], and only the key points relevant to this review will be highlighted

Correspondence to:
Raymond Lai, MD, PhD
Department of Laboratory Medicine and Pathology, Cross Cancer Institute and University of Alberta, Rm 2338, Cross Cancer Institute, 11560 University Avenue, Edmonton, Alberta, Canada T6G 1Z2
rlai@ualberta.ca

Robert J. Ingham, PhD
Department of Medical Microbiology and Immunology, Li Ka Shing Institute of Virology, University of Alberta, Edmonton, Alberta, Canada

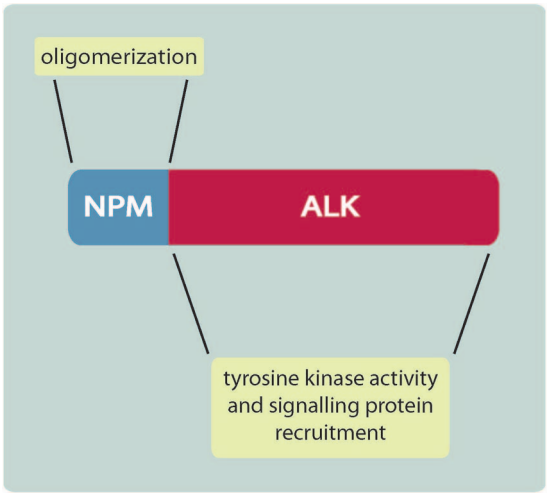

Figure 1. ALK, anaplastic lymphoma kinase; NPM, nucleophosmin.

here. The t(2;5)(p23;q35) chromosomal translocation and the resulting fusion protein NPM-ALK are found in the majority of ALK+ALCL, which represents a small subset of T-cell non-Hodgkin lymphoma recognized as a distinct pathologic entity in the World Health Organization classification scheme [Delsol *et al.* 2008]. Structurally, NPM-ALK is composed of a short portion of the N-terminus of NPM which contains the oligomerization domain; this segment is fused with the C-terminal tyrosine kinase domain and intracellular tail of the ALK protein [Morris *et al.* 1994] (Figure 1). The oligomerization domain of NPM provides the biochemical basis for the NPM-ALK proteins to undergo oligomerization, which is a requirement for the tyrosine auto-phosphorylation and constitutive activation of NPM-ALK [Fujimoto *et al.* 1996; Iwahara *et al.* 1997].

The expression of the full-length ALK, a cell-surface receptor carrying a tyrosine kinase domain in its cytoplasmic portion, is normally restricted to the brain and nervous system [Iwahara *et al.* 1997; Pulford *et al.* 1997]. Normal lymphocytes do not express ALK. In ALK+ALCL cells, the expression of *NPM-ALK* is driven by the promoter of *NPM* which is ubiquitously expressed [Wang *et al.* 1993]. As a consequence of this abnormal gene fusion, the tyrosine kinase activity of ALK is constitutively active, and exerting its biological effects in a 'foreign' cell type.

Studies investigating how NPM-ALK promotes oncogenesis have turned out to be highly rewarding in the field of cancer biology, and NPM-ALK has proven to be an excellent study model for understanding how oncogenic tyrosine kinases work. As summarized in the previous review papers, NPM-ALK is known to promote tumorigenecity by binding to and constitutively activating a host of cellular signaling proteins, including those in the signaling pathways of signal transducer and activator of transcription 3 (STAT3), MEK/ERK, mammalian target of rapamycin (mTOR) and phosphoinositide 3 kinase (PI3K)/Akt [Amin and Lai, 2007; Chiarle *et al.* 2008; Palmer *et al.* 2009; Inghirami and Pileri, 2011]. The activation of these pathways results in increased cell proliferation and resistance to apoptosis.

The NPM-ALK/STAT3 signaling axis

One of the better characterized pathways deregulated by NPM-ALK is that of STAT3 (Figure 2). It has been shown that NPM-ALK promotes the tyrosine phosphorylation of STAT3 on one of its tyrosine residues, Y705, which is critical for the dimerization and activation of STAT3 [Zamo *et al.* 2002; Zhang *et al.* 2002]. Generally, activated STAT3 proteins migrate to the nucleus, where they function as transcription factors in the regulation of a large number of genes [Levy and Darnell, 2002]. STAT3 activation, as evidenced by the expression of phospho-STAT3 and the nuclear localization of the STAT3 protein detectable by immunohistochemistry, is indeed a marker consistently expressed by ALK+ALCL tumors [Zamo *et al.* 2002; Zhang *et al.* 2002; Khoury *et al.* 2003]. Inhibition of STAT3 using a dominant negative construct was shown to induce effective apoptosis and cell-cycle arrest in ALK+ALCL cell lines [Amin *et al.* 2004]. The

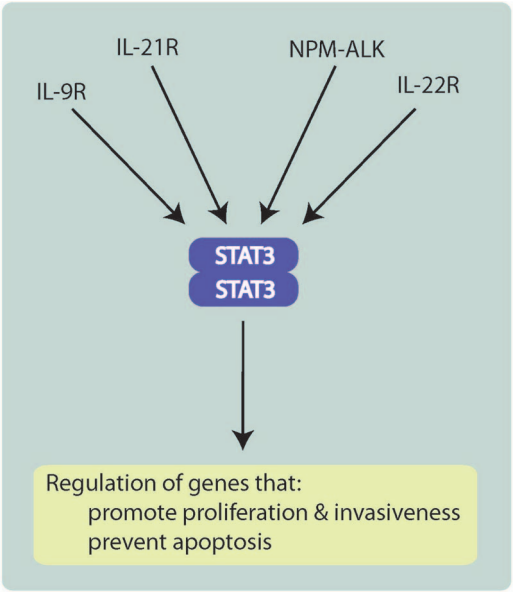

Figure 2. ALK, anaplastic lymphoma kinase; IL, interleukin; NPM, nucleophosmin; STAT3, signal transducer and activator of transcription 3.

importance of STAT3 in ALK+ALCL is further highlighted by the study published by Chiarle and colleagues, in which STAT3 was shown to be required in the setting of NPM-ALK-induced lymphomagensis [Chiarle *et al.* 2005]. Constitutive activation of STAT3 is associated with the upregulation of many proteins associated with cell proliferation and survival [Zamo *et al.* 2002; Amin *et al.* 2004; Chiarle *et al.* 2005; Piva *et al.* 2006, 2010; Anastasov *et al.* 2010; Zhang *et al.* 2011a], and this appears to be true for other cancer cell types in which STAT3 is constitutively active.

Additional studies of how STAT3 promotes the tumorigenesis of ALK+ALCL have revealed novel insights. One example is the observation that STAT3 functionally interacts with DNA methyltransferases, and thereby promotes promoter hypermethylation and gene silencing [Zhang *et al.* 2005, 2011b]. One gene is *SHP1*, which encodes a tyrosine phosphatase that negatively regulates the STAT3 signaling pathway [Zhang *et al.* 2005; Han *et al.* 2006a, 2006b; Honorat *et al.* 2006]. Previous studies have shown that SHP1 promoter methylation and loss of SHP1 expression are indeed frequently observed in ALK+ALCL cell lines and tumors [Khoury *et al.* 2004]. Functional studies have supported that loss of *SHP1* is pathogenetically important in ALK+ALCL, as restoration of SHP1 by gene transfection inhibits the growth of these cells [Han *et al.* 2006a; Hegazy *et al.* 2010]. Interestingly, restoration of SHP1 not only dampens the level of STAT3 activation, it also promotes proteosomal degradation of NPM-ALK [Han *et al.* 2006a]. Thus, through silencing SHP1 expression, the NPM-ALK/STAT3 signaling axis is sustained and enhanced.

Accumulating evidence supports that constitutive activation of STAT3 in ALK+ALCL is multifactorial, a concept that was first brought forward by Zhang and colleagues [Zhang *et al.* 2002]. Further support for this concept came when it was found that JAK3, a tyrosine kinase that normally relays cytokine stimulation to STAT3 activation, is constitutively activated in ALK+ALCL cell lines and tumors [Zamo *et al.* 2002; Amin *et al.* 2003; Lai *et al.* 2005]. In ALK+ALCL cells, JAK3 coimmunoprecipitates with NPM-ALK and STAT3, and it appears to directly enhance the tyrosine phosphorylation/activation status of both proteins [Amin *et al.* 2003]. In this same study, small interfering RNA (siRNA) knockdown or pharmacologic inhibition of JAK3 resulted in significant apoptosis in ALK+ALCL cells, supporting the notion that JAK3 is biologically important in this lymphoma.

Since the JAK proteins are normally activated via cytokine stimulation, the finding of JAK3 activation in ALK+ALCL cells raises the possibility that cytokine stimulation may contribute to the activation status of NPM-ALK and STAT3, and thus, the tumorigenecity of ALK+ALCL. JAK3 is

activated by interleukins of the interleukin (IL)-2 common γ chain family (IL-2Rγ), which includes IL-2, IL4, IL-7, IL-9, IL15 and IL-21 [Witthuhn *et al.* 1994; Yin *et al.* 1995; Asao *et al.* 2001; Habib *et al.* 2002]. Our research team demonstrated that ALK+ALCL cell lines produce both IL-9 and IL-21 [Qiu *et al.* 2006; Dien Bard *et al.* 2009]. Moreover, we provided evidence that these two cytokines promote tumorigenesis by activating JAK3/STAT3 signaling via an autocrine stimulatory loop. In keeping with our conclusion that IL-9 is important in the context of NPM-ALK–driven lymphomagenesis, overexpression of NPM-ALK in IL-9 transgenic mice was found to induce lymphoma formation [Lange *et al.* 2003].

Intriguingly, in a recently published study, it was reported that STAT3 promotes the binding of DNA methyltransferases to the *IL-2Rγ* promoter so as to repress the expression of *IL-2Rγ* [Zhang *et al.* 2011b]. These findings certainly challenge the notion that IL-9 and IL-21 autocrine stimulatory loops contribute to the pathogenesis of ALK+ALCL, as the signaling of these two cytokines is IL-2Rγ dependent [Kimura *et al.* 1995; Asao *et al.* 2001; Habib *et al.* 2002]. While this discrepancy cannot be fully explained without further experiments, one possibility may be related to the use of different cell clones of ALK+ALCL cell lines by different laboratories. The finding by Zhang and colleagues that stimulation with IL-2, IL-9 and IL-15 did not stimulate STAT3 even in ALK+ALCL cells transfected with the *IL-2Rγ* gene suggests that these cell clones are intrinsically insensitive to these cytokines [Zhang *et al.* 2011b].

In addition to IL-9 and IL-21, our group found that IL-22 also contributes to STAT3 activation in ALK+ALCL cells [Dien Bard *et al.* 2008]. In contrast to the signaling of IL-9 and IL-21, IL-22 signaling is independent of IL-2Rγ. Unlike the receptors for IL-9 and IL-21, IL-22R1 (which is one of the subunits of the IL-22 receptor) is absent in normal lymphocytes [Wolk *et al.* 2010]. Thus, the expression of IL-22R1 on the cytoplasmic membrane of ALK+ALCL cells is an aberrant event. Results from *in vitro* studies support that IL-22 signaling contributes to the pathogenesis of ALK+ALCL, as inhibition of the signaling of this pathway using either IL-22 neutralizing antibodies or naturally occurring IL-22 decoy receptors (e.g. IL-22BP) decreased STAT3 activation and significantly inhibited cell growth and colony formation in soft agar; opposite biological effects were observed when recombinant IL-22 was added to ALK+ALCL cells [Dien Bard *et al.* 2008]. Interestingly, we also found that the aberrant expression of IL-22R1 is directly attributed to NPM-ALK, as gene transfection of *NPM-ALK c*DNA into Jurkat cells induced IL-22R1 expression. Thus, NPM-ALK can convert an 'IL-22–unresponsive' phenotype to an 'IL-22-responsive' phenotype. The functional relationship among NPM-ALK, STAT3 and IL-22 signaling also illustrates an excellent example by which NPM-ALK contributes to STAT3 activation via an alternative pathway.

Most recently, our laboratories described yet another mechanism by which STAT3 activation can be enhanced in ALK+ALCL. We found that β catenin, a transcriptional factor and the downstream mediator of the Wnt canonical pathway, is constitutively active in ALK+ALCL cells [Anand *et al.* 2011]. Downregulation of β catenin using siRNA resulted in a marked reduction in the STAT3 levels and activated/phosphorylated STAT3. While NPM-ALK did not regulate the protein level, nuclear localization or tyrosine phosphorylation of β catenin in ALK+ALCL cells, it upregulated β-catenin transcriptional activity via a yet-to-be defined mechanism. These observations regarding the relationship between β catenin and NPM-ALK are in contrast with that between β catenin and BCR-ABL, an oncogenic tyrosine kinase expressed in chronic myeloid leukemia. In these cells, BCR-ABL contributes to the stabilization of β catenin by increasing its tyrosine phosphorylation, nuclear translocation and transcriptional activity [Coluccia *et al.* 2007].

In normal cells, β catenin is inhibited by glycogen synthase kinase 3β (GSK3β), which phosphorylates β catenin at multiple serine/threonine residues and thereby promotes its proteosomal degradation [Jope and Johnson, 2004]. GSK3β itself can be inhibited by the PI3K/Akt and the Wnt canonical pathways [Manoukian and Woodgett, 2002]. Specifically, inactivation of GSK3β is mediated via the phosphorylation of its serine 9 residue. Upon inactivation, its inhibitory effect on β catenin is released, and β catenin is allowed to accumulate and translocate to the nucleus, where it upregulates the transcription of multiple genes that promote cell growth. In a recent report, it has been shown that NPM-ALK promotes the phosphorylation of GSK3β on serine 9, likely through PI3K/Akt signaling [McDonnell *et al.* 2011]. This study further demonstrated that inhibition of GSK3β allowed its substrates, Mcl1 and CDC25A, to accumulate in ALK+ALCL cells.

Considering the role of active GSK3β in targeting β catenin for degradation, we believe that these results correlate well with our findings regarding the constitutive activation of β catenin in ALK+ALCL cells.

It has been shown that STAT3 activation contributes to the growth of ALK+ALCL cells by upregulating a large number of genes promoting cell-cycle progression and inhibiting apoptosis, and these details have been summarized previously [Amin and Lai, 2007; Chiarle *et al.* 2008; Palmer *et al.* 2009; Inghirami and Pileri, 2011]. More recent studies have revealed that the mechanisms by which STAT3 mediates the oncogenic effects of NPM-ALK are more diverse than originally thought. In this regard, the interaction between STAT3 and DNA methytransferases leads to gene silencing of SHP1 as was discussed earlier. STAT3-mediated epigenetic silencing is also responsible for the loss of T-cell signaling molecules, including CD3ε, ZAP70, LAT and SLP76, all of which are important in determining T-cell identity and T-cell receptor signaling [Ambrogio *et al.* 2009]. A recent paper has revealed that STAT3 binds to the promoter of hypoxia-induced factor 1α (HIF1α) and upregulates HIF1α expression in ALK+ALCL cells [Marzec *et al.* 2011]. This finding suggests that HIF1α could represent an important mechanism by which STAT3 exerts its oncogenic effects in ALK+ALCL, as HIF1α is known to be involved in tumor growth, angiogenesis, invasiveness, metastasis and drug resistance in other types of malignancy [Majmundar *et al.* 2010; Rohwer and Cramer, 2011]. The same research group also reported that STAT3 in ALK+ALCL upregulates the mRNA and protein expression of CD274 (PD-L1, B7-H1), which is a cell-surface protein implicated in immunosuppression [Marzec *et al.* 2008]. To be discussed below, STAT3 has been shown to modulate the expression of specific microRNAs (miRNAs) in this type of lymphoma.

Our laboratories have recently published that STAT3 substantially upregulates Twist1, a protein well known for its role in promoting epithelial mesenchymal transition and the metastatic potential of solid tumors [Yang *et al.* 2004]. We found that ALK+ALCL cell lines and tumors consistently express a high level of Twist1; in contrast, normal T cells do not have detectable Twist1. Results from our *in vitro* invasion assays support that Twist1 contributes to the invasiveness of ALK+ALCL cells, and this biological response correlated with the activation of Akt and downregulation of p66Shc [Zhang *et al.* 2012]. Importantly, the knockdown of Twist1 expression in ALK+ALCL results in increased sensitivity to PF-2341066 (Crizotinib; Pfizer, New York, NY)), which is a US Food and Drug Administration approved ALK inhibitor.

Most recently, our group has found that the NPM-ALK/STAT3 signaling axis strongly upregulates the Sox-2 (sex-determining region Y-box-2) transcription factor (Gelebart *et al.* 2012). The significance of this finding is underlined by the fact that the major function of Sox-2 in normal cells is to promote self-renewal and pluripotency of embryonic stem cells [Niwa *et al.* 2000; Chew *et al.* 2005]. Furthermore, Sox-2 represents one of the few transcription factors involved in the reprogramming and conversion of terminally differentiated fibroblasts into inducible pluripotent stem cells [Park *et al.* 2008; Okita and Yamanaka, 2010]. Using confocal microscopy, the expression of the Sox-2 protein was detectable in virtually all of the cells in the two ALK+ALCL cell lines tested. However, the transcriptional activity of Sox-2 appears to be restricted to a relatively small subset of cells. Importantly, the subset with active Sox-2 activity was more tumorigenic and invasive than cells without Sox2 activity. While the biological role of Sox-2 in ALK+ALCL still needs to be further examined, Sox-2 represents the latest member of downstream targets of the NPM-ALK/STAT3 axis.

Mass spectrometry studies: identifying novel pathways that mediate NPM-ALK-mediated tumorigenesis

Thus far, we have discussed the role of STAT3 as a key mediator for NPM-ALK-induced tumorigenesis. However, to fully understand the pathobiology of NPM-ALK, several laboratories including ours have performed extensive mass spectrometry studies to profile proteins that are physically bound to NPM-ALK [Crockett *et al.* 2004; Galietta *et al.* 2007; Wu *et al.* 2009]. Results from these studies have proven to be highly useful in unveiling novel oncogenic signaling functions of NPM-ALK. One example is the identification of MSH2 as an NPM-ALK-binding protein [Wu *et al.* 2009]. MSH2 is a protein known to be pivotal for DNA mismatch repair (MMR). Specifically, MSH2 heterodimerizes with MSH6 (known as MutSα) or MSH3 (as known as MutSβ), to detect single base mismatches and small insertion–deletion loops [Li, 2008]. Once the MSH2 heterodimers bind to the site of DNA error, a second

MMR heterodimer consisting of MutL orthologs is recruited (e.g. MLH1•PMS2), followed by the recruitment of additional proteins that mediate the removal of the erroneous DNA bases, using the unaffected strand as a template to resynthesize the DNA [Li, 2008]. Cells lacking MSH2 can form neither MutSα nor MutSβ, and are completely deficient in MMR function [Reitmair *et al.* 1995; Andrew *et al.* 1998; Edelmann and Edelmann, 2004]. Failure of MMR function, such as in the loss of MSH2, results in a mutator phenotype and subsequent tumorigenesis [Reitmair *et al.* 1995]. The importance of MMR function to tumor suppression has been characterized most extensively in Lynch syndrome, an early onset cancer syndrome affecting a heterogeneous group of tissues (e.g. colon, endometrium, urothelium). In addition, acquired inactivation of MMR contributes to a proportion of sporadic cancers [Coleman and Tsongalis, 2001]. Pertinent to this review, the congenital loss of MMR function through the inheritance of two mutated copies of a gene encoding a MMR protein, including MSH2, is associated with the development of lymphoid cancer presenting in childhood [Felton *et al.* 2007].

Based on this background, the finding that MSH2, but not its normal binding partners, MSH3 and MSH6, binds to NPM-ALK led us to hypothesize that NPM-ALK may interfere with MMR, by virtue of sequestrating MSH2 away from MSH3 and MSH6. In support of this hypothesis, we found that gene transfection of *NPM-ALK* cDNA into GP293 cells indeed significantly impaired MMR, as measured by 6-thioguanine sensitivity [Young *et al.* 2011]. Furthermore, site-directed mutagenesis of tyrosine 191 on NPM-ALK, significantly disrupted NPM-ALK•MSH2 binding and partially restored 6-thioguanine sensitivity. Lastly, we also found that NPM-ALK promoted tyrosine phosphorylation of MSH2, which might be a critical step for its functional interference of MSH2 and MMR function. While further experiments are needed to understand this phenomenon more completely, MSH2 represents a novel target of NPM-ALK, and interference of the MMR represents a novel oncogenic function of this fusion protein. Interestingly, it has been reported that another oncogenic tyrosine kinase, BCR-ABL, can interfere with MMR *in vitro* [Stoklosa *et al.* 2008]. It is tempting to speculate that BCR-ABL may also interact with MSH2 in a similar manner as NPM-ALK.

Mass spectrometry data have also revealed an interaction between NPM-ALK and various heat shock proteins (Hsps) [Crockett *et al.* 2004; Boccalatte *et al.* 2009; Wu *et al.* 2009]. Most studies regarding Hsps in the context of NPM-ALK pathobiology have been focused on Hsp90, which is a molecular chaperone that plays a critical role in helping proteins, termed clients, fold correctly. There are a multitude of Hsp90 clients in cells and they include protein kinases, transcription factors and chromatin remodeling factors (reviewed by Taipale and colleagues and Trepel and colleagues) [Taipale *et al.* 2010; Trepel *et al.* 2010]. A number of tyrosine kinases critical in oncogenesis including Bcr-Abl [An *et al.* 2000], Her2/Neu [Citri *et al.* 2002], Flt3 [Minami *et al.* 2002] and Raf-1 [Stancato *et al.* 1993] are known Hsp90 clients. Moreover, the inhibition of Hsp90 with benzoquinone ansamycin drugs, such as herbimycin A, geldanamycin and related compounds such as 17-allylamino,17-demethoxygeldanamycin (17-AAG) impairs the activity of these kinases [Okabe *et al.* 1992; Stancato *et al.* 1993; Miller *et al.* 1994; Schulte *et al.* 1995; An *et al.* 2000; Naoe *et al.* 2001; Citri *et al.* 2002; Minami *et al.* 2002]. These drugs bind the adenosine triphosphate binding amino-terminus of Hsp90 and interfere with client protein interaction, which ultimately results in client protein degradation [Holzbeierlein *et al.* 2010]. Several studies have implicated NPM-ALK as an important Hsp90 client.

Treatment of ALK+ALCL cell lines with Hsp90 inhibitors resulted in decreased NPM-ALK expression, and this is most likely due to the targeting of this protein for proteasomal degradation [Bonvini *et al.* 2002; Georgakis *et al.* 2006]. Previous work by Bonvini and colleagues demonstrated that disruption of the interaction between NPM-ALK and Hsp90, as a result of 17-AAG treatment, correlated with an increase in the association of the Hsp70 chaperone protein with NPM-ALK [Bonvini *et al.* 2004]. Hsp70 complexes with the CHIP E3 ubiquitin ligase, and Hsp70/CHIP complexes targeted NPM-ALK for proteasomal degradation in 17-AAG-treated cells [Bonvini *et al.* 2004]. Furthermore, the treatment of ALK+ALCL cell lines with 17-AAG results in cell cycle arrest in G_0/G_1 and an increase in apoptosis [Georgakis *et al.* 2006]. It should be noted that the biological effect of Hsp90 inhibition in ALK+ALCL is likely not exclusively due to inhibition of NPM-ALK, as Hsp90 has many client proteins. For example, Hsp90 has also been shown to be important for regulating the activity of matrix metalloproteinase 9 in ALK+ALCL and allowing these cells to invade through matri-gel [Lagarrigue *et al.* 2010]. Our group has also recently shown that the

Hsp90 co-chaperone, cyclophilin 40, is regulated by NPM-ALK signaling and promotes the viability of ALK+ ALCL cell lines [Pearson *et al*. 2012].

In view of the fact that NPM-ALK is a constitutively active tyrosine kinase, identifying proteins whose phosphorylation status is influenced by NPM-ALK would be expected to provide insights into the pathobiology of this oncoprotein. To that end, mass spectrometry studies were performed by several laboratories, including ours, focusing on the phosphoproteomic changes induced by NPM-ALK [Rush *et al.* 2005; Boccalatte *et al.* 2009; Wu *et al.* 2010]. In order to optimize the sensitivity and specificity of our assay, we employed an approach combining sequential affinity purification of phosphopeptides and liquid chromatography–tandem mass spectrometry. We identified a large number of phosphoproteins that were differentially expressed in lysates of GP293 cells transfected with NPM-ALK compared with GP293 cells transfected with a kinase-dead NPM-ALK [Wu *et al.* 2010]. Specifically, 506 phosphoproteins (617 phosphopeptides and 767 phosphorylation sites) were identified. Our method was designed to purify all phosphopeptides, and thus, we were able to detect a large number of serine/threonine phosphorylated proteins [Wu *et al.* 2010].

Our phosphoproteomic studies have revealed several other pathways that were not previously known to be regulated by NPM-ALK. For instance, we found evidence of tyrosine phosphorylation of multiple proteins in the tumor necrosis factor (TNF) signaling pathway. Using western blotting, we validated that the induction of the tyrosine phosphorylation of several TNF pathway signaling molecules by NPM-ALK, including RIP, TRAP1 and FAF1 [Wu *et al.* 2010]. In support of their functional importance in this type of lymphoma, we knocked down the expression of TRAP1 using siRNA, and found that this treatment significantly sensitized ALK+ALCL cell lines to TRAIL- and doxorubicin-induced apoptosis. We also found that NPM-ALK induced the phosphorylation of multiple proteins in the ubiquitin–proteasome degradation pathway, including proteasome activator 28 subunit γ (PSME3) [Wu *et al.* 2010]. These findings echo results from a previous mass spectrometry study we performed, in which we found that NPM-ALK associates with at least six E3 ubiquitin ligases [Wu *et al.* 2009].

Interestingly, NPM-ALK itself showed evidence of serine phosphorylation [Wang *et al.* 2011]. Using tandem affinity purification mass spectrometry, we found evidence of phosphorylation of three serine residues of NPM-ALK (serine 135, 164 and 497) when ectopically expressed in GP293 cells. Phosphorylation of these residues is functionally important, as site-directed mutagenesis of these sites led to a significant reduction in the tumorigenecity of NPM-ALK in this experimental model. Thus, although the oncogenic activity of NPM-ALK is dependent on its tyrosine autophosphorylation, phosphorylation of its serine residues appears to enhance tumorigenic potential. While we have confirmed that NPM-ALK in ALK+ALCL tumors is serine phosphorylated, it remains to be determined whether NPM-ALK in tumors is actually phosphorylated on the same serine residues (i.e. serine 135, 164 and 497) as in ALK+ALCL cell lines.

The regulation of miRNAs in ALK+ALCL

miRNAs are small (~20 nt) nonprotein-coding RNAs that regulate diverse cellular functions. These molecules bind the 3' untranslated region of mRNAs, usually resulting in either translational repression or degradation of the targeted mRNA [Fabian *et al.* 2010]. miRNAs are appreciated to play an important role in many cancers [Kasinski and Slack, 2011], and this includes ALK+ALCL. A recent study has even demonstrated that NPM-ALK itself is targeted by miRNA 96 [Vishwamitra *et al*. 2012]. In addition, several studies have demonstrated that NPM-ALK signaling represses or promotes the expression of miRNAs that influence the cellular phenotype and pathogenesis of ALK+ ALCL.

In a study by Desjobert and colleagues, the repression of miR-29a was found to be dependent on NPM-ALK activity and STAT3, and this repression is likely mediated by the epigenetic silencing of miR-29a [Desjobert *et al.* 2011]. The importance of miR-29a repression in ALK+ALCL was demonstrated by experiments showing that ectopic expression of this miRNA in ALK+ ALCL cell lines resulted in decreased protein levels of the antiapoptotic protein, Mcl-1, a miR-29a target. Decreased Mcl-1 expression correlated with an increased sensitivity of cells to doxorubicin. This study also showed that immunocompromised mice injected with miR-29a-expressing Karpas 299 cells formed smaller tumors that were more apoptotic than tumors formed in mice injected with Karpas 299 cells expressing a control miRNA.

Several other miRNAs are repressed by NPM-ALK and STAT3 signaling in ALK+ALCL. This includes miR-21 and miR-219, which in this lymphoma target DNA methyltransferase 1 [Zhang *et al.* 2011b] and the proliferation promoting receptor ICOS [Zhang *et al.* 2011a], respectively. Dejean and colleagues demonstrated that miR-16 was significantly downregulated in cells isolated from the lymph nodes of mice expressing an inducible NPM-ALK or TPM3-ALK transgene compared with cells isolated from mice in which the transgene was not induced [Dejean *et al.* 2011]. Surprisingly, however, this group found that treatment of TPM3-ALK-expressing mouse embryo fibroblasts with Crizotinib did not increase miR-16 expression and even slightly decreased miR-16 levels. This raises the question as to whether this miRNA is indeed regulated by NPM-ALK. Regardless, the overexpression of miR-16 was found to decrease expression of the VEGF growth factor in ALK+ALCL cell lines and an inverse correlation between miR-16 and VEGF expression was observed in patient samples [Dejean *et al.* 2011]. Moreover, miR-16 injection in the tumors of nude mice was also found to decrease tumor growth *in vivo* [Dejean *et al.* 2011]. miR-101 was also found to be downregulated in ALK+ALCL cell lines, but whether this miRNA is regulated by NPM-ALK signaling was not examined [Merkel *et al.* 2010]. The forced expression of miR-101 in ALK+ALCL cell lines was found to result in an increased number of cells arrested in G1 as well as an increase in apoptosis. These phenotypes are likely a result of the downregulation of the miR-101 targets, the serine/threonine kinase mTOR and the prosurvival protein Mcl-1 [Merkel *et al.* 2010].

NPM-ALK signaling also leads to the upregulation of several important miRNAs in ALK+ALCL. For example, miR-135b was found to be highly expressed in ALK+ALCL cell lines and patient samples [Lawrie *et al.* 2008; Matsuyama *et al.* 2011], and its expression was NPM-ALK and STAT3 dependent [Matsuyama *et al.* 2011]. Matsuyama and colleagues further showed that miR-135b targets the FOXO1 transcription factor in ALK+ALCL cell lines, which is critical as FOXO1 can promote the expression of the cell cycle inhibitors p21 and p27. Moreover, this group showed that miR-135b is responsible for IL-17 production in this lymphoma, conferring a 'Th17-like' immunophenotype to tumor cells.

Future directions

Since the discovery of the NPM-ALK fusion protein in 1994, significant progress has been made with respect to our understanding of the oncogenic impact and properties of NPM-ALK. Nevertheless, a number of questions remain to be addressed. For instance, the functional importance of serine/threonine phosphorylation of NPM-ALK needs to be further clarified. It is also unclear as to whether the pathogenesis of pediatric ALK+ALCL cases is similar to that involved in adult cases. Lastly, as more cancer types are found to possess other ALK translocations as well as ALK mutations, it has yet to be fully established whether we can extrapolate what we have learned from the studies of NPM-ALK in ALK+ALCL to these malignancies.

Funding

Research in the Lai Laboratory is supported by operating research grants from the Canadian Institute of Health Research, the Canadian Cancer Society and the Alberta Cancer Foundation. Research in the Ingham laboratory is supported by the Alberta Cancer Foundation.

Conflict of interest statement

The authors have no conflicting financial interests.

References

Ambrogio, C., Martinengo, C., Voena, C., Tondat, F., Riera, L., Di Celle, P. *et al.* (2009) Npm-Alk oncogenic tyrosine kinase controls T-cell identity by transcriptional regulation and epigenetic silencing in lymphoma cells. Cancer Res 69: 8611–8619.

Amin, H. and Lai, R. (2007) Pathobiology of Alk+ anaplastic large-cell lymphoma. *Blood* 110: 2259–2267.

Amin, H., McDonnell, T., Ma, Y., Lin, Q., Fujio, Y., Kunisada, K. *et al.* (2004) Selective inhibition of STAT3 induces apoptosis and G(1) cell cycle arrest in ALK-positive anaplastic large cell lymphoma. *Oncogene* 23: 5426–5434.

Amin, H., Medeiros, L., Ma, Y., Feretzaki, M., Das, P., Leventaki, V. *et al.* (2003) Inhibition of JAK3 induces apoptosis and decreases anaplastic lymphoma kinase activity in anaplastic large cell lymphoma. *Oncogene* 22: 5399–407.

An, W., Schulte, T. and Neckers, L. (2000) The heat shock protein 90 antagonist geldanamycin alters chaperone association with P210BCR-ABL and V-Src

proteins before their degradation by the proteasome. *Cell Growth Differ* 11: 355–360.

Anand, M., Lai, R. and Gelebart, P. (2011) Beta-catenin is constitutively active and increases STAT3 expression/activation in anaplastic lymphoma kinase-positive anaplastic large cell lymphoma. *Haematologica* 96: 253–261.

Anastasov, N., Bonzheim, I., Rudelius, M., Klier, M., Dau, T., Angermeier, D. *et al.* (2010) C/EBPβ expression in ALK-positive anaplastic large cell lymphomas is required for cell proliferation and is induced by the STAT3 signaling pathway. *Haematologica* 95: 760–767.

Andrew, S., Mckinnon, M., Cheng, B., Francis, A., Penney, J., Reitmair, A. *et al.* (1998) Tissues of MSH2-deficient mice demonstrate hypermutability on exposure to a DNA methylating agent. *Proc Natl Acad Sci U S A* 95: 1126–1130.

Asao, H., Okuyama, C., Kumaki, S., Ishii, N., Tsuchiya, S., Foster, D. *et al.* (2001) Cutting edge: the common gamma-chain is an indispensable subunit of the IL-21 receptor complex. *J Immunol* 167: 1–5.

Azarova, A., Gautam, G. and George, R. (2011) Emerging importance of ALK in neuroblastoma. *Semin Cancer Biol* 21: 267–275.

Boccalatte, F., Voena, C., Riganti, C., Bosia, A., D'Amico, L., Riera, L. *et al.* (2009) The enzymatic activity of 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase is enhanced by NPM-ALK: new insights in ALK-mediated pathogenesis and the treatment of ALCL. *Blood* 113: 2776–2790.

Bonvini, P., Dalla Rosa, H., Vignes, N. and Rosolen, A. (2004) Ubiquitination and proteasomal degradation of nucleophosmin-anaplastic lymphoma kinase induced by 17-allylamino-demethoxygeldanamycin: role of the co-chaperone carboxyl heat shock protein 70-interacting protein. *Cancer Res* 64: 3256–3264.

Bonvini, P., Gastaldi, T., Falini, B. and Rosolen, A. (2002) Nucleophosmin-anaplastic lymphoma kinase (NPM-ALK), a novel HSP90-client tyrosine kinase: down-regulation of NPM-ALK expression and tyrosine phosphorylation in ALK(+) CD30(+) lymphoma cells by the HSP90 antagonist 17-allylamino,17-demethoxygeldanamycin. *Cancer Res* 62: 1559–1566.

Chew, J., Loh, Y., Zhang, W., Chen, X., Tam, W., Yeap, L. *et al.* (2005) Reciprocal transcriptional regulation of Pou5f1 and Sox2 via the Oct4/Sox2 complex in embryonic stem cells. *Mol Cell Biol* 25: 6031–6046.

Chiarle, R., Simmons, W., Cai, H., Dhall, G., Zamo, A., Raz, R. *et al.* (2005) STAT3 is required for ALK-mediated lymphomagenesis and provides a possible therapeutic target. *Nat Med* 11: 623–629.

Chiarle, R., Voena, C., Ambrogio, C., Piva, R. and Inghirami, G. (2008) The anaplastic lymphoma kinase in the pathogenesis of cancer. *Nat Rev Cancer* 8: 11–23.

Citri, A., Alroy, I., Lavi, S., Rubin, C., Xu, W., Grammatikakis, N. *et al.* (2002) Drug-induced ubiquitylation and degradation of ErbB receptor tyrosine kinases: implications for cancer therapy. *EMBO J* 21: 2407–2417.

Coleman, W. and Tsongalis, G. (2001) The role of genomic instability in the development of human cancer. In: Coleman, W. and Tsongalis, G. (eds), *The Molecular Basis of Human Cancer*, 1st ed. Humana Press, Totowa, NJ: pp. 115–142.

Coluccia, A., Vacca, A., Dunach, M., Mologni, L., Redaelli, S., Bustos, V. *et al.* (2007) BCR-ABL stabilizes beta-catenin in chronic myeloid leukemia through its tyrosine phosphorylation. *EMBO J* 26: 1456–1466.

Crockett, D., Lin, Z., Elenitoba-Johnson, K. and Lim, M. (2004) Identification of NPM-ALK interacting proteins by tandem mass spectrometry. *Oncogene* 23: 2617–2629.

Crystal, A. and Shaw, A. (2011) New targets in advanced NSCLC: EML4-ALK. *Clin Adv Hematol Oncol* 9: 207–214.

Dejean, E., Renalier, M., Foisseau, M., Agirre, X., Joseph, N., De Paiva, G. *et al.* (2011) Hypoxia-microrna-16 downregulation induces VEGF expression in anaplastic lymphoma kinase (Alk)-positive anaplastic large-cell lymphomas. *Leukemia* 25: 1882–1890.

Delsol, G., Falini, B., Muller-Hermelink, H., Campo, E., Jaffe, E., Gascoyne, R. *et al.* (2008) *Anaplastic Large Cell Lymphoma (ALCL), ALK-positive*. 4th ed. Lyon: International Agency for Research on Cancer (IARC).

Desjobert, C., Renalier, M., Bergalet, J., Dejean, E., Joseph, N., Kruczynski, A. *et al.* (2011) MiR-29a down-regulation in ALK-positive anaplastic large cell lymphomas contributes to apoptosis blockade through MCL-1 overexpression. *Blood* 117: 6627–6637.

Dien Bard, J., Gelebart, P., Anand, M., Amin, H. and Lai, R. (2008) Aberrant expression of IL-22 receptor 1 and autocrine IL-22 stimulation contribute to tumorigenicity in ALK+ anaplastic large cell lymphoma. *Leukemia* 22: 1595–1603.

Dien Bard, J., Gelebart, P., Anand, M., Zak, Z., Hegazy, S., Amin, H. *et al.* (2009) IL-21 contributes to JAK3/STAT3 activation and promotes cell growth in ALK-positive anaplastic large cell lymphoma. *Am J Pathol* 175: 825–834.

Edelmann, L. and Edelmann, W. (2004) Loss of DNA mismatch repair function and cancer predisposition in the mouse: animal models for human hereditary nonpolyposis colorectal cancer. *Am J Med Genet C Semin Med Genet* 129C: 91–99.

Fabian, M., Sonenberg, N. and Filipowicz, W. (2010) Regulation of MRNA translation and stability by micrornas. *Annu Rev Biochem* 79: 351–379.

Felton, K., Gilchrist, D. and Andrew, S. (2007) Constitutive deficiency in DNA mismatch repair. *Clin Genet* 71: 483–498.

Fujimoto, J., Shiota, M., Iwahara, T., Seki, N., Satoh, H., Mori, S. *et al.* (1996) Characterization of the transforming activity of p80, a hyperphosphorylated protein in a Ki-1 lymphoma cell line with chromosomal translocation t(2;5). *Proc Natl Acad Sci U S A* 93: 4181–4186.

Galietta, A., Gunby, R., Redaelli, S., Stano, P., Carniti, C., Bachi, A. *et al.* (2007) NPM/ALK binds and phosphorylates the RNA/DNA-binding protein PSF in anaplastic large-cell lymphoma. *Blood* 110: 2600–2609.

Gelebart, P., Hegazy, S.A., Wang, P., Bone, K.M., Anand, M., Sharon, D. *et al.* (2012) Aberrant expression and biological significance of Sox2, an embryonic stem cell transcriptional factor, in ALK-positive anaplastic large cell lymphoma. *Blood Cancer Journal* 2: e82.

Georgakis, G., Li, Y., Rassidakis, G., Medeiros, L. and Younes, A. (2006) The HSP90 inhibitor 17-AAG synergizes with doxorubicin and U0126 in anaplastic large cell lymphoma irrespective of ALK expression. *Exp Hematol* 34: 1670–1679.

Habib, T., Senadheera, S., Weinberg, K. and Kaushansky, K. (2002) The common gamma chain (gamma C) is a required signaling component of the IL-21 receptor and supports IL-21-induced cell proliferation via JAK3. *Biochemistry* 41: 8725–8731.

Han, Y., Amin, H., Franko, B., Frantz, C., Shi, X. and Lai, R. (2006a) Loss of SHP1 enhances JAK3/STAT3 signaling and decreases proteosome degradation of JAK3 and NPM-ALK in ALK+ anaplastic large-cell lymphoma. *Blood* 108: 2796–2803.

Han, Y., Amin, H., Frantz, C., Franko, B., Lee, J., Lin, Q. *et al.* (2006b) Restoration of SHP1 expression by 5-AZA-2'-deoxycytidine is associated with downregulation of JAK3/STAT3 signaling in ALK-positive anaplastic large cell lymphoma. *Leukemia* 20: 1602–1609.

Hegazy, S., Wang, P., Anand, M., Ingham, R., Gelebart, P. and Lai, R. (2010) The tyrosine 343 residue of nucleophosmin (NPM)-anaplastic lymphoma kinase (ALK) is important for its interaction with SHP1, a cytoplasmic tyrosine phosphatase with tumor suppressor functions. *J Biol Chem* 285: 19813–19820.

Holzbeierlein, J., Windsperger, A. and Vielhauer, G. (2010) HSP90: a drug target? *Curr Oncol Rep* 12: 95–101.

Honorat, J., Ragab, A., Lamant, L., Delsol, G. and Ragab-Thomas, J. (2006) SHP1 tyrosine phosphatase negatively regulates NPM-ALK tyrosine kinase signaling. *Blood* 107: 4130–4138.

Inghirami, G. and Pileri, S. (2011) Anaplastic large-cell lymphoma. *Semin Diagn Pathol* 28: 190–201.

Iwahara, T., Fujimoto, J., Wen, D., Cupples, R., Bucay, N., Arakawa, T. *et al.* (1997) Molecular characterization of ALK, a receptor tyrosine kinase expressed specifically in the nervous System. *Oncogene* 14: 439–449.

Jope, R. and Johnson, G. (2004) The glamour and gloom of glycogen synthase kinase-3. *Trends Biochem Sci* 29: 95–102.

Kasinski, A. and Slack, F. (2011) Epigenetics and genetics. Micrornas en route to the clinic: progress in validating and targeting micrornas for cancer therapy. *Nat Rev Cancer* 11: 849–864.

Khoury, J., Medeiros, L., Rassidakis, G., Yared, M., Tsioli, P., Leventaki, V. *et al.* (2003) Differential expression and clinical significance of tyrosine-phosphorylated STAT3 in ALK+ and ALK- anaplastic large cell lymphoma. *Clin Cancer Res* 9: 3692–3699.

Khoury, J., Rassidakis, G., Medeiros, L., Amin, H. and Lai, R. (2004) Methylation of SHP1 gene and loss of SHP1 protein expression are frequent in systemic anaplastic large cell lymphoma. *Blood* 104: 1580–1581.

Kimura, Y., Takeshita, T., Kondo, M., Ishii, N., Nakamura, M., Van Snick, J. *et al.* (1995) Sharing of the IL-2 receptor gamma chain with the functional IL-9 receptor complex. *Int Immunol* 7: 115–120.

Lagarrigue, F., Dupuis-Coronas, S., Ramel, D., Delsol, G., Tronchere, H., Payrastre, B. *et al.* (2010) Matrix metalloproteinase-9 is upregulated in nucleophosmin-anaplastic lymphoma kinase-positive anaplastic lymphomas and activated at the cell surface by the chaperone heat shock protein

90 to promote cell invasion. *Cancer Res* 70: 6978–6987.

Lai, R., Rassidakis, G., Lin, Q., Atwell, C., Medeiros, L. and Amin, H. (2005) JAK3 activation is significantly associated with ALK expression in anaplastic large cell lymphoma. *Hum Pathol* 36: 939–944.

Lange, K., Uckert, W., Blankenstein, T., Nadrowitz, R., Bittner, C., Renauld, J. *et al.* (2003) Overexpression of NPM-ALK induces different types of malignant lymphomas in IL-9 transgenic mice. *Oncogene* 22: 517–527.

Lawrie, C., Saunders, N., Soneji, S., Palazzo, S., Dunlop, H., Cooper, C. *et al.* (2008) MicroRNA expression in lymphocyte development and malignancy. *Leukemia* 22: 1440–1446.

Levy, D. and Darnell, J., Jr (2002) Stats: transcriptional control and biological impact. *Nat Rev Mol Cell Biol* 3: 651–662.

Li, G. (2008) Mechanisms and functions of DNA mismatch repair. *Cell Res* 18: 85–98.

Majmundar, A., Wong, W. and Simon, M. (2010) Hypoxia-inducible factors and the response to hypoxic stress. *Mol Cell* 40: 294–309.

Manoukian, A. and Woodgett, J. (2002) Role of glycogen synthase kinase-3 in cancer: regulation by Wnts and other signaling pathways. *Adv Cancer Res* 84: 203–229.

Marzec, M., Liu, X., Wong, W., Yang, Y., Pasha, T., Kantekure, K. *et al.* (2011) Oncogenic kinase NPM/ALK induces expression of HIF1α mRNA. *Oncogene* 30: 1372–1378.

Marzec, M., Zhang, Q., Goradia, A., Raghunath, P., Liu, X., Paessler, M. *et al.* (2008) Oncogenic kinase NPM/ALK induces through STAT3 expression of immunosuppressive protein CD274 (PD-L1, B7-H1). *Proc Natl Acad Sci U S A* 105: 20852–20857.

Matsuyama, H., Suzuki, H., Nishimori, H., Noguchi, M., Yao, T., Komatsu, N. *et al.* (2011) miR-135b Mediates NPM-ALK-driven oncogenicity and renders IL-17-producing immunophenotype to anaplastic large cell lymphoma. *Blood* 118: 6881–6892.

McDonnell, S., Hwang, S., Basrur, V., Conlon, K., Fermin, D., Wey, E. *et al.* (2011) NPM-ALK signals through glycogen synthase kinase 3β to promote oncogenesis. *Oncogene* 31: 3733-3740.

Merkel, O., Hamacher, F., Laimer, D., Sifft, E., Trajanoski, Z., Scheideler, M. *et al.* (2010) Identification of differential and functionally active miRNAs in both anaplastic lymphoma kinase (ALK)+ and ALK-anaplastic large-cell lymphoma. *Proc Natl Acad Sci U S A* 107: 16228–16233.

Miller, P., Diorio, C., Moyer, M., Schnur, R., Bruskin, A., Cullen, W. *et al.* (1994) Depletion of the erbB-2 gene product p185 by benzoquinoid ansamycins. *Cancer Res* 54: 2724–2730.

Minami, Y., Kiyoi, H., Yamamoto, Y., Yamamoto, K., Ueda, R., Saito, H. *et al.* (2002) Selective apoptosis of tandemly duplicated FLT3-transformed leukemia cells by HSP90 inhibitors. *Leukemia* 16: 1535–1540.

Morris, S., Kirstein, M., Valentine, M., Dittmer, K., Shapiro, D., Saltman, D. *et al.* (1994) Fusion of a kinase gene, ALK, to a nucleolar protein gene, NPM, in non-Hodgkin's lymphoma. *Science* 263: 1281–1284.

Naoe, T., Kiyoe, H., Yamamoto, Y., Minami, Y., Yamamoto, K., Ueda, R. *et al.* (2001) FLT3 tyrosine kinase as a target molecule for selective antileukemia therapy. *Cancer Chemother Pharmacol* 48(Suppl. 1): S27–S30.

Niwa, H., Miyazaki, J. and Smith, A. (2000) Quantitative expression of Oct-3/4 defines differentiation, dedifferentiation or self-renewal of ES cells. *Nat Genet* 24: 372–376.

Ogawa, S., Takita, J., Sanada, M. and Hayashi, Y. (2011) Oncogenic mutations of ALK in neuroblastoma. *Cancer Sci* 102: 302–308.

Okabe, M., Uehara, Y., Miyagishima, T., Itaya, T., Tanaka, M., Kuni-Eda, Y. *et al.* (1992) Effect of herbimycin A, an antagonist of tyrosine kinase, on bcr/abl oncoprotein-associated cell proliferations: abrogative effect on the transformation of murine hematopoietic cells by transfection of a retroviral vector expressing oncoprotein P210bcr/Abl and preferential inhibition on Ph1-positive leukemia cell growth. *Blood* 80: 1330–1338.

Okita, K. and Yamanaka, S. (2010) Induction of pluripotency by defined factors. *Exp Cell Res* 316: 2565–2570.

Palmer, R., Vernersson, E., Grabbe, C. and Hallberg, B. (2009) Anaplastic lymphoma kinase: signalling in development and disease. *Biochem J* 420: 345–361.

Park, I., Zhao, R., West, J., Yabuuchi, A., Huo, H., Ince, T. *et al.* (2008) Reprogramming of human somatic cells to pluripotency with defined factors. *Nature* 451: 141–146.

Pearson, J.D., Mohammed, Z., Bacani, J.T., Lai, R. and Ingham, R.J. (2012) The heat shock protein-90 co-chaperone, Cyclophilin 40, promotes ALK-positive, anaplastic large cell lymphoma viability and its expression is regulated by the NPM-ALK oncoprotein. *BMC Cancer* 12.

Piva, R., Agnelli, L., Pellegrino, E., Todoerti, K., Grosso, V., Tamagno, I. *et al.* (2010)

Gene expression profiling uncovers molecular classifiers for the recognition of anaplastic large-cell lymphoma within peripheral T-cell neoplasms. *J Clin Oncol* 28: 1583–1590.

Piva, R., Pellegrino, E., Mattioli, M., Agnelli, L., Lombardi, L., Boccalatte, F. *et al.* (2006) Functional validation of the anaplastic lymphoma kinase signature identifies CEBPB and Bcl2A1 as critical target genes. *J Clin Invest* 116: 3171–3182.

Pulford, K., Lamant, L., Morris, S., Butler, L., Wood, K., Stroud, D. *et al.* (1997) Detection of anaplastic lymphoma kinase (ALK) and nucleolar protein nucleophosmin (NPM)-ALK proteins in normal and neoplastic cells with the monoclonal antibody ALK1. *Blood* 89: 1394–1404.

Qiu, L., Lai, R., Lin, Q., Lau, E., Thomazy, D., Calame, D. *et al.* (2006) Autocrine release of interleukin-9 promotes JAK3-dependent survival of ALK+ anaplastic large-cell lymphoma cells. *Blood* 108: 2407–2415.

Reitmair, A., Schmits, R., Ewel, A., Bapat, B., Redston, M., Mitri, A. *et al.* (1995) MSH2 deficient mice are viable and susceptible to lymphoid tumours. *Nat Genet* 11: 64–70.

Rohwer, N. and Cramer, T. (2011) Hypoxia-mediated drug resistance: novel insights on the functional interaction of HIFs and cell death pathways. *Drug Resist Updat* 14: 191–201.

Rush, J., Moritz, A., Lee, K., Guo, A., Goss, V., Spek, E. *et al.* (2005) Immunoaffinity profiling of tyrosine phosphorylation in cancer cells. *Nat Biotechnol* 23: 94–101.

Sasaki, T. and Janne, P. (2011) New strategies for treatment of ALK-rearranged non-small cell lung cancers. *Clin Cancer Res* 17: 7213–7218.

Schulte, T., Blagosklonny, M., Ingui, C. and Neckers, L. (1995) Disruption of the Raf-1-Hsp90 molecular complex results in destabilization of Raf-1 and loss of Raf-1-Ras association. *J Biol Chem* 270: 24585–24588.

Shiota, M., Fujimoto, J., Semba, T., Satoh, H., Yamamoto, T. and Mori, S. (1994) Hyperphosphorylation of a novel 80 kDa protein-tyrosine kinase similar to Ltk in a human Ki-1 lymphoma cell line, AMS3. *Oncogene* 9: 1567–1574.

Stancato, L., Chow, Y., Hutchison, K., Perdew, G., Jove, R. and Pratt, W. (1993) Raf exists in a native heterocomplex with Hsp90 and p50 that can be reconstituted in a cell-free system. *J Biol Chem* 268: 21711–21716.

Stoklosa, T., Poplawski, T., Koptyra, M., Nieborowska-Skorska, M., Basak, G., Slupianek, A. *et al.* (2008) BCR/ABL inhibits mismatch repair to protect from apoptosis and induce point mutations. Cancer Research 68: 2576–2580.

Taipale, M., Jarosz, D. and Lindquist, S. (2010) HSP90 at the hub of protein homeostasis: emerging mechanistic insights. *Nat Rev Mol Cell Biol* 11: 515–528.

Trepel, J., Mollapour, M., Giaccone, G. and Neckers, L. (2010) Targeting the dynamic HSP90 complex in cancer. *Nat Rev Cancer* 10: 537–549.

Vishwamitra, D., Li, Y., Wilson, D., Manshouri, R., Curry, C.V., Shi, B. *et al.* (2012) MicroRNA 96 is a post-transcriptional suppressor of anaplastic lymphoma kinase expression. *American Journal of Pathology* 180: 1772–1780.

Wang, D., Umekawa, H. and Olson, M. (1993) Expression and subcellular locations of two forms of nucleolar protein B23 in rat tissues and cells. *Cell Mol Biol Res* 39: 33–42.

Wang, P., Wu, F., Zhang, J., McMullen, T., Young, L., Ingham, R. *et al.* (2011) Serine phosphorylation of NPM-ALK, which is dependent on the auto-activation of the kinase activation loop, contributes to its oncogenic potential. *Carcinogenesis* 32: 146–153.

Witthuhn, B., Silvennoinen, O., Miura, O., Lai, K., Cwik, C., Liu, E. *et al.* (1994) Involvement of the JAK-3 Janus kinase in signalling by interleukins 2 and 4 in lymphoid and myeloid cells. *Nature* 370: 153–157.

Wolk, K., Witte, E., Witte, K., Warszawska, K. and Sabat, R. (2010) Biology of interleukin-22. *Semin Immunopathol* 32: 17–31.

Wu, F., Wang, P., Young, L., Lai, R. and Li, L. (2009) Proteome-wide identification of novel binding partners to the oncogenic fusion gene protein, NPM-ALK, using tandem affinity purification and mass spectrometry. *Am J Pathol* 174: 361–370.

Wu, F., Wang, P., Zhang, J., Young, L., Lai, R. and Li, L. (2010) Studies of phosphoproteomic changes induced by nucleophosmin-anaplastic lymphoma kinase (ALK) highlight deregulation of tumor necrosis factor (TNF)/Fas/TNF-related apoptosis-induced ligand signaling pathway in ALK-positive anaplastic large cell lymphoma. *Mol Cell Proteomics* 9: 1616–1632.

Yang, J., Mani, S., Donaher, J., Ramaswamy, S., Itzykson, R., Come, C. *et al.* (2004) Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. *Cell* 117: 927–939.

Yin, T., Yang, L. and Yang, Y. (1995) Tyrosine phosphorylation and activation of JAK family tyrosine linases by interleukin-9 in MO7E cells. *Blood* 85: 3101–3106.

Young, L., Bone, K., Wang, P., Wu, F., Adam, B., Hegazy, S. *et al.* (2011) Fusion tyrosine kinase NPM-ALK deregulates MSH2 and suppresses

DNA mismatch repair function novel insights into a potent oncoprotein. *Am J Pathol* 179: 411–421.

Zamo, A., Chiarle, R., Piva, R., Howes, J., Fan, Y., Chilosi, M. *et al.* (2002) Anaplastic lymphoma linase (ALK) Activates STAT3 and protects hematopoietic cells from cell death. *Oncogene* 21: 1038–1047.

Zhang, J., Wang, P., Wu, F., Li, M., Sharon, D., Ingham, R. *et al.* (2012) Aberrant expression of the transcriptional factor Twist1 promotes invasiveness in ALK-positive anaplastic large cell lymphoma. *Cell Signal* 24: 852–858.

Zhang, Q., Raghunath, P., Xue, L., Majewski, M., Carpentieri, D., Odum, N. *et al.* (2002) Multilevel dysregulation of STAT3 activation in anaplastic lymphoma kinase-positive T/null-cell lymphoma. *J Immunol* 168: 466–474.

Zhang, Q., Wang, H., Kantekure, K., Paterson, J., Liu, X., Schaffer, A. *et al.* (2011a) Oncogenic tyrosine kinase NPM-ALK induces expression of the growth-promoting receptor ICOS. *Blood* 118: 3062–3071.

Zhang, Q., Wang, H., Liu, X., Bhutani, G., Kantekure, K. and Wasik, M. (2011b) IL-2R common gamma-chain is epigenetically silenced by nucleophosphin-anaplastic lymphoma kinase (NPM-ALK) and acts as a tumor suppressor by targeting NPM-ALK. *Proc Natl Acad Sci U S A* 108: 11977–11982.

Zhang, Q., Wang, H., Marzec, M., Raghunath, P., Nagasawa, T. and Wasik, M. (2005) STAT3- and DNA methyltransferase 1-mediated epigenetic silencing of SHP-1 tyrosine phosphatase tumor suppressor gene in malignant T lymphocytes. *Proc Natl Acad Sci U S A* 102: 6948–6953.