Comment on Boccalatte et al, page 2776

Unraveling ALK signaling through phosphoproteomics

Megan S. Lim UNIVERSITY OF MICHIGAN

In this issue of *Blood*, Boccalatte and colleagues report on innovative mass spectrometry–driven proteomic studies designed to determine the effect of the NPM-ALK oncogenic tyrosine kinase on the phosphoproteomic-signaling pathway of ALCL.1

The t(2;5)(p23;q35) chromosomal aberration resulting in over-expression of a chimeric oncogene, nucleophosmin–anaplastic lymphoma kinase (NPM-ALK),2 is the most common translocation found in anaplastic large cell lymphoma (ALCL). The constitutive activation of the oncogenic ALK tyrosine kinase induces signaling events that lead to the neoplastic phenotype of ALCL. Numerous signaling pathways downstream of NPM-ALK have been identified including phospholipase Cγ (PLCγ), phosphatidylinositol-3-kinase (PI3K), RAC-serine/threonine-protein kinase (AKT), signal transducer and activator of transcription 3 (STAT3), and the nonreceptor protein kinase (SRC).3 However, most of these studies have been using hypothesis-driven approaches that lead to the identification of the role of a single protein or pathway. Furthermore, analysis of the activation state of a protein as implied by phosphorylation status or sites of phosphorylations are limited to those for which phosphorylation–specific antibodies exist.

Large scale unbiased approaches limited to the transcriptional signature have been used to identify “ALK” target genes, however, the translation of these data sets to signaling proteins induced by a tyrosine kinase may have limited biologic relevance. Advances in liquid chromatography–tandem mass spectrometry–driven proteomics have shifted the paradigm of translational cancer research that enables fast and reliable large scale study of proteins involved in many disease models.4 It follows that identifying the ALK-regulated proteome5,6 and phosphoproteome would lead to key insights into the signaling pathways important for the pathogenesis of ALCL.

Using quantitative proteomic–based approaches coupled with an enrichment strategy that allow the identification of not just the phosphorylated peptides/proteins but specific phosphorylation sites within the peptides,7 the authors present a large dataset of phosphoproteins in 6 cell lines derived from ALCLs and alterations that occur in response to NPM-ALK shRNA or ALK kinase inhibitors. A common ALK phosphotyrosine signature, which included both up-regulation of phosphoproteins and down-regulation of other phosphoproteins, was identified using complementary approaches including small molecular ALK inhibitor (CEP14083) and shRNA. The power of this technology is demonstrated by the observation that of the proteins that are exclusively identified in ALK + cells, 5-aminoimidazole-4-carboxamide ribonucleotide formyltransferase/IMP cyclohydrolase (ATIC) is phosphorylated at Y104. The authors provide functional validation of the role of ATIC and demonstrate that its phosphorylation is regulated by NPM/ALK. The biologic relevance of ALK-mediated ATIC activity is highlighted by the observation that the transformylase and total enzyme activity of ATIC is reduced by exposure to methotrexate and could confer resistance to the drug.

These studies not only add to the complexity of downstream signaling pathways induced by ALK but raise the potential for other approaches that enable “activity profiling” of other posttranslational mechanisms to study ALK signaling. These large scale proteomic approaches will have clinical implications in other ALK-deregulated cancers, such as lung cancer8 and neuroblastoma.9

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES

1. Boccalatte FE, Voena C, Riganti C, et al. 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. 2009;113:2776-2790.
PHAGOCYTES & GRANULOCYTES

Comment on Ohnmacht and Voehringer, page 2816

The expanding universe of the basophil

Michael F. Gurish  Brigham and Women’s Hospital

In this issue of Blood, Ohnmacht and Voehringer expand our view of the murine basophil, implicating these cells in eosinophilia and intestinal helminth rejection as well as determining the lifespan of basophils in both basal and inflammatory states.

Interest in the role of basophils in immune responses has been rekindled recently. Demonstration of their ability to promote humoral memory responses, drive IgE-dependent dermatitis, and shift an immune response toward TH-2–dominated inflammation are some of the many biologic activities described. In this issue, Ohnmacht and Voehringer address the development and lifespan of basophils and further investigate their role in helminth infections. In addressing these issues, they first identified the expression of the Thy1 antigen on the surface of IL-4–expressing cells, which were defined by expression of green fluorescent protein in the 4get transgenic mouse. The basophils were distinguished from the other IL-4+ FceRIε cell type, the mast cell, by the lack of the mast cell marker c-kit. They then used the Thy-1 antigen to deplete these cells in mice by the injection of an anti-Thy1 antibody. Other labs have previously used antibody directed to FceRIε or CD200R3. The depletion of basophils by anti-Thy1 represents a novel approach, albeit limited by the fact that due to the expression of this antigen by T cells, it was only useful in lymphocyte-deficient RAG-/- mice that lack T and B cells and are used to study only innate immune responses. Nonetheless, basophil-depleted RAG-/- mice clearly showed a diminished capacity to reject Nippostrongylus worms from the small intestine and decreased eosinophilia in both the lung and spleen. Further, using passive sensitization followed by antigen activation, they noted increased numbers of eosinophils in blood, spleen, and lung, and an increase in IL-5 message in the lung. As both of these effects were Thy-1 sensitive, this suggests IL-5 production by the activated basophils is important in promoting and maintaining the attendant eosinophilia in these tissues which in turn likely mediated the helminth expulsion.

Unfortunately, all of these strategies to deplete basophils suffer from the possibility of off-target effects which have not been fully addressed. In all 3 instances, the markers used for depletion are also expressed on the mast cell, the committed mast cell progenitor, and/or the common basophil-mast cell progenitor identified in the spleen of C57BL/6 mice by Arinobu et al. Although none of the authors noted a decrease in the mature mast cell number identified in the peritoneal cavity of mice, these cells represent a mature end-stage cell that may not be affected in the limited time frame of these treatments. Nonetheless, this consideration does not diminish other aspects of the study, which demonstrated a life span of basophiles of only about 60 hours and the increased production of these cells in the bone marrow following helminth infection. This latter finding is consistent with those of Arinobu et al who, following infection with the helminth, Trichinella spiralis, also noted increased basophil production by the bone marrow and increased numbers of the common basophil–mast cell progenitor in the spleen. Importantly, Ohnmacht and Voehringer then addressed whether the increased numbers associated with TH-2 inflammation was the result of increased production, increased survival, or both. The increased survival as a consequence of the cytokines generated by TH-2 inflammation, particularly IL-3, has been shown in vitro using human basophils and was recently further studied by Didichenko et al, who found this is mediated by IL-3 activation of PIM-1. To test whether the basophil lifespan in vivo was increased in association with inflammation provoked by the Nippostrongylus infection, the authors transferred labeled basophils and noted the rate of loss of these cells in normal versus infected mice. No difference in clearance of the cells was noted between the 2 groups of animals, indicating that the increase in basophil number is likely due to the increased production of these cells in the bone marrow. In lieu of the availability of a basophil-deficient mouse to confirm the findings, these studies represent the best approach and provide a significant increase in our understanding of the development and expanding role of these cells in inflammation.

Conflict-of-interest disclosure: The author declares no competing financial interests.

REFERENCES

1. Denezel A, Maus UA, Gomez MR, et al. Basophils enhance immunological memory responses. Nat Immunol. 2008;9:733-742.
2. Obata K, Mukai K, Tsuburaya Y, et al. Basophils are essential initiators of a novel type of chronic allergic inflammation. Blood. 2007;110:913-920.
3. Hida S, Tadachi M, Saito T, et al. Negative control of mast cell development and expanding role of these cells in innate immunity. Int Arch Allergy Immunol. 1992;97:322-329.
4. Didichenko SA, Spiegl N, Brunner T, et al. IL-3 induces a Pim1-dependent antiapoptotic pathway in primary human basophils. Blood. 2008;112:3949-3958.

2616 19 MARCH 2009 | VOLUME 113, NUMBER 12
Unraveling ALK signaling through phosphoproteomics

Megan S. Lim