

## Editorial

# Single Cell Phospho-Signaling Analysis in Myeloid Malignancies

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Abnormal hematopoietic stem/progenitor cell (HSPC) signaling is a hallmark of leukemic hematopoiesis and a common denominator in the pathobiology of all types of myeloid malignancies. The most studied pathways are the JAK/STAT, MAPK/ERK and PI3K/AKT cascades [1]. Constitutive activation of STAT molecules has been reported in almost all myeloid malignancies. Basal STAT3 and less often STAT5 levels are encountered in acute myeloid leukemia (AML) blasts and have been associated with worse outcome [2], whereas STAT1 is sporadically overexpressed in AML and CML and its role in leukemogenesis is unclear [3]. The central role of the JAK/STAT network in clonal myelopoiesis is typically illustrated in Ph- myeloproliferative neoplasms (MPN). The activating V617F mutation of JAK2 kinase is encountered in all MPN types, whereas it has been recently suggested that the antagonism between STAT1 and STAT5 activation in CD34<sup>+</sup> cells determines the final phenotype of a JAK2V617F-positive MPN [4].

The other two signaling pathways playing pivotal roles in cell proliferation, differentiation and survival are the MAPK/ERK and PI3/AKT cascades [1]. Both pathways target a wide array of downstream molecules with diverse biological functions and have long been implicated in tumorigenesis [5]. Conditional activation of MAPK/ERK pathway is also observed in a variety of myeloid neoplasms [5] potentially conferring adverse outcomes [6]. Similarly constitutively phosphorylated AKT is frequently present in AML, while MAPK/ERK and PI3/AKT pathways can also synergize to induce leukemic transformation of hematopoietic cell lines [7].

However, the above studies were performed with conventional proteomic techniques and were mainly focused on the constitutive expression of one or a few signaling molecules in often heterogeneous cell populations, rather than looking at unified pathways. Signaling cascades of hematopoiesis are largely regulated by cytokines and altered growth factor responses of signaling networks of leukemic cells are equally frequent with constitutive activation of specific molecules. Thus, assessing only the constitutive levels of various signaling modules in myeloid malignancies does not capture the overall picture of signaling events at the single cell level and begets limited pathophysiological insights and contradictory prognostic information [6,8].

Single cell network profiling (SCNP) is a novel, flow cytometry based approach, which enables simultaneous measurements of both basal phosphorylation and responses to extracellular modulators of a plethora of signaling molecules [9,10]. By using SCNP, a wide range of critical cellular processes involved in leukemic transformation can be investigated synchronously at the single cell level [11]. This is of great importance for many reasons. First, by testing the signaling response to various stimulators SCNP helps to elucidate how malignant cells process signals from their environment, thus providing important mechanistic insights [9,12]. Second, in contrast to the limited prognostic value of conventional surface immunophenotyping in myeloid neoplasms [13,14], functional phenotyping of clonal cells by SCNP may identify distinct progenitor cell subsets with more aggressive behavior and/or resistance to both chemotherapy and hypomethylating agents [12,15]. More important, patients harboring the same mutation may display differential activity of the associated signaling pathway which correlates with the clinical phenotype [4,15], emphasizing that signaling profiles are not merely a surrogate for the underlying molecular abnormalities, but instead provide additional information. Third, the information taken by SCNP can be translated in various clinical applications, such as development of targeted therapies, monitoring chemotherapy responses and early cancer detection. Therefore, SCNP, particularly reinforced by the advent of the sophisticated approach of single-mass cytometry [16], might be able to unravel the Ariadne's thread of the extreme phenotypic heterogeneity of myeloid neoplasms by drafting patient-specific rather than disease-specific signaling biosignatures. The constantly accumulating biological and prognostic information presages a time where personalized therapy in myeloid malignancies may be potentially customized on the basis of individual signaling profiles.

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