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Time resolved quantitative phospho-tyrosine analysis reveals Bruton's tyrosine kinase mediated signaling downstream of the mutated granulocyte-colony stimulating factor receptors

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Granulocyte-colony stimulating factor receptor (G-CSFR) controls myeloid progenitor proliferation and differentiation to neutrophils. Mutations in CSF3R (encoding G-CSFR) have been reported in patients with chronic neutrophilic leukemia (CNL) and acute myeloid leukemia (AML); however, despite years of research, the malignant downstream signaling of the mutated G-CSFRs is not well understood. Here, we used a quantitative phospho-tyrosine analysis to generate a comprehensive signaling map of G-CSF induced tyrosine phosphorylation in the normal versus mutated (proximal: T618I and truncated: Q741x) G-CSFRs. Unbiased clustering and kinase enrichment analysis identified rapid induction of phospho-proteins associated with endocytosis by the wild type G-CSFR only; while G-CSFR mutants showed abnormal kinetics of canonical Stat3, Stat5, and MAPK phosphorylation, and aberrant activation of Bruton's tyrosine kinase (Btk). Mutant-G-CSFR-expressing cells displayed enhanced sensitivity (3–5-fold lower IC50) for ibrutinib-based chemical inhibition of Btk. Primary murine progenitor cells from G-CSFR-Q741x knock-in mice validated activation of Btk by the mutant receptor and retrovirally transduced human CD34+ umbilical cord blood cells expressing mutant receptors displayed enhanced sensitivity to ibrutinib. A significantly lower clonogenic potential was displayed by both murine and human primary cells expressing mutated receptors upon ibrutinib treatment. Finally, a dramatic synergy was observed between ibrutinib and ruxolinitib at lower dose of the individual drug. Altogether, these data demonstrate the strength of unsupervised proteomics analyses in dissecting oncogenic pathways, and suggest repositioning ibrutinib for therapy of myeloid leukemia bearing CSF3R mutations. Phospho-tyrosine data are available via ProteomeXchange with identifier PXD009662.

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