New study paves the way for precision drugs to treat blood cancers

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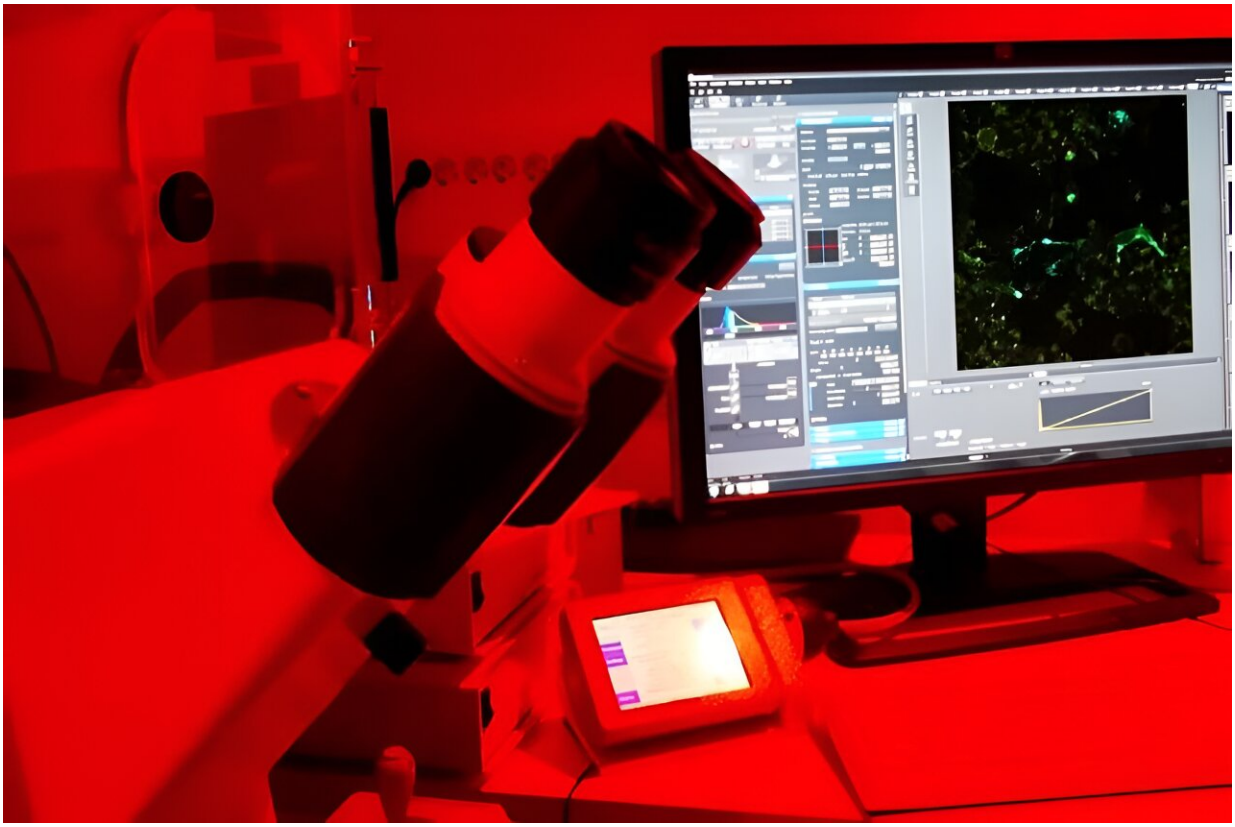

Receptor dimerization is assayed by fluorescence microscopy at Tampere Imaging Facility. Credit: Antti Kurttila

The Janus kinase 2 (JAK2) protein mediates signaling from several cytokine receptors in the regulation of hematopoiesis and immune

responses. Somatic mutations in human JAK2 lead to constitutive activation and cytokine-independent signaling and underlie several hematological malignancies from myeloproliferative neoplasms (MPN) to acute leukemia and lymphomas. JAK2 contains an active kinase domain and an inactive pseudokinase domain. Interestingly, pathogenic mutations mainly occur in the regulatory pseudokinase domain.

Due to its critical pathogenic role, JAK2 has become an important therapeutic target. The four currently approved JAK2 inhibitors relieve symptoms but do not heal the patient or affect survival. These drugs target the highly conserved kinase domain and affect both normal and mutated JAK2 and, due to side effects, carry a black box warning that limits their use in elderly, cardiac and cancer patients. The selective inhibition of pathogenic JAK2 is a key pending goal in drug discovery that requires a precise mechanistic understanding of the regulation of JAK2 activation.

"To understand the molecular and structural basis of the physiological and pathogenic activation of JAK2, we used single-molecule microscopy and erythropoietin receptor (EpoR) as a model system.

"In contrast to several previous studies, we showed that the JAK2-EpoR complex is not a preformed dimer. Instead, JAK2 activation proceeds via the ligand-induced dimerization of EpoR monomers," says Academy Research Fellow Teemu Haikarainen from Tampere University.

JAK2-EpoR dimerization is a common mechanism for normal and pathogenic activation by JAK2 mutations. Importantly, the new study discovered that all major JAK2 mutation types—exon 12 (causing polycythemia vera), V617F (80% of all three types of MPN), and exon 16 (acute lymphoid leukemia)—utilize pseudokinase domain-mediated JAK2 dimerization as a mechanism for pathogenic activation.

The more detailed analysis of the pathogenic activation mechanisms was achieved by a combination of X-ray crystallography, molecular dynamic simulations and AI-guided modeling. The analyses revealed different pseudokinase dimerization interfaces for the pathogenic mutants and provided an explanation for their distinct activation mechanisms.

Furthermore, the studies indicated that the single amino-acid mutations in the pseudokinase domain cause different conformations in full-length JAK2 that may explain their differing clinical presentations.

"The results significantly extend our understanding of normal and pathogenic JAK2 activation. This project started 30 years ago when we cloned the JAK2 gene and showed its function in cytokine signaling. Our subsequent studies have focused on the pseudokinase domain and discovered its regulatory function in cytokine signaling, and importantly, demonstrated the pseudokinase domain as a valid drug target.

"We believe that the novel structural insights on the molecular changes in mutation driven JAK2 hyperactivation open now new possibilities in the selective targeting of the pseudokinase domain and pathogenic JAK2 signaling in different disease entities," says Professor Olli Silvennoinen from Tampere University.

The research article was published in *Science Advances*, "Molecular basis of JAK2 activation in erythropoietin receptor and pathogenic JAK2 signaling," and authored by Bobin George Abraham, Teemu Haikarainen, Joni Vuorio, Mykhailo Girych, Anniina T. Virtanen, Antti Kurttila, Christos Karathanasis, Mike Heilemann, Vivek Sharma, Ilpo Vattulainen and Olli Silvennoinen.

More information: Bobin George Abraham et al, Molecular basis of

JAK2 activation in erythropoietin receptor and pathogenic JAK2 signaling, *Science Advances* (2024). DOI: 10.1126/sciadv.adl2097