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## 602.MYELOID ONCOGENESIS: BASIC

### Intracellular IL-23 Receptor (IL-23R) Is a Regulator of Mitotic Spindle and Centrosome Formation and Is Essential for AML Viability

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IL-23 receptor (IL-23R) is a heterodimeric cytokine cell surface receptor that is traditionally expressed on T cells. The cytokine, IL-23, is secreted by dendritic cells and macrophages in response to inflammatory stimuli. Here, we identify a novel function and localization for IL-23R in which intracellular IL-23R is necessary for AML viability and acts as a critical regulator of the mitotic spindle.

We analyzed gene ontologies that were upregulated in AML samples compared to normal hematopoietic cells. Amongst upregulated gene ontologies, the mitotic spindle ontology was enriched in AML samples. Further analysis of all protein coding genes and their correlation to the mitotic spindle ontology surprisingly returned IL-23R as a top hit. Given that IL-23R is not known to be expressed in AML, we confirmed the presence of IL-23R protein by immunoblotting in 14 of 20 primary AML patient samples, while it was undetectable in bulk (n=5) and CD34+ sorted (n=3) normal hematopoietic cells.

Consistent with previous investigations, we confirmed cell surface localization of IL-23R in double negative T cells by flow cytometry and confocal microscopy. In contrast, only small amounts of IL-23R were present on the cell surface of AML cells. Instead, IL-23R was detected intracellularly in the cytoplasm and nucleus of AML cell lines, as well as primary AML cells (including the stem cell fraction). We demonstrated intracellular localization using flow cytometry, confocal microscopy, and immunoblotting of subcellular fractions. To ensure we were detecting genuine IL-23R protein, we probed for IL-23R using 4 different antibodies targeting 4 different epitopes of the receptor whilst using 4 methods of detection (immunoblotting, flow cytometry, confocal microscopy, and immunoprecipitation). We also demonstrated intracellular localization of the IL-23R heterodimer subunit, IL12R$\beta$1, which is known to bind the IL-23R subunit to form the fully functional IL-23 receptor.

To elucidate the function of intracellular IL-23R, we performed BioID mass spectrometry to identify proteins that interact with IL-23R. Compared to controls, we identified 61 proteins that preferentially interacted with IL-23R. 36 of those 61 proteins are known cytoplasmic or nuclear localized proteins. Pathway analysis of those interacting proteins identified the mitotic spindle as a top pathway corroborating with our bioinformatics analysis. Proximity Ligation Assay (PLA) and confocal microscopy verified that endogenous IL-23R protein interacted with mitotic spindle associated proteins, NUMA, TMEM201, TACC1, and BAG6 in OCI-AML2 cells and primary AML samples. Consistent with our PLA results, we demonstrated IL-23R co-localized with the mitotic spindle and centrosomes in AML cell lines and primary patient samples. Knockdown and knockout of IL-23R in AML cells led to the dysregulation of the mitotic spindle with multipolarity, lagging chromosomes, and spindle orientation errors.

Knockdown of IL-23R reduced cell proliferation and viability in OCI-AML2, TEX, K562, NB4, and U937 cells compared to non-targeting controls. IL-23R knockdown also reduced the engraftment efficiency of TEX leukemia cells in murine bone marrow.

Subsequent knockdowns of IL-23R in primary AML cells demonstrated decreased clonogenic growth and reduced engraftment into the marrow of immune deficient mice. In contrast, knockdown of IL-23R in normal human cord blood cells did not impair their engraftment into murine bone marrow. Finally, we analyzed hematopoietic cells and stem cells in IL-23R -/- mice and found that knockout of IL-23R did not decrease blood counts or the abundance and function of normal hematopoietic cells.

In summary, we discovered a novel intracellular localization and function for IL-23R in AML. IL-23R regulates mitotic spindle and centrosome formation and is necessary for AML cell viability. We have thus identified a new biological function for IL-23R and a potential therapeutic target for AML.

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