No.	K24-1116	
Project Title	Deciphering and targeting EZH2 non-canonical activities to overcome therapy resistance in acute myeloid leukemia.	
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(Form 2)

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IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual)

Annual Report

Report

Background & Research objectives

Epigenetic alterations play a major role in cancer, making epigenetic regulators promising therapeutic targets. Among them, EZH2 is a key enzyme with a well-established role in carcinogenesis, yet its mechanisms of action remain poorly understood. Notably, EZH2 can act either as a tumor suppressor or as an oncogene depending on the tumor type. This dual role may be explained by variations in its activity according to tissue and cellular context.
Beyond its canonical function as part of the PRC2 complex, where it catalyzes the repressive H3K27me3 mark, EZH2 also exhibits non-canonical ("EZH2-solo") activities that could account for its pleiotropic effects. Understanding these distinct functions is essential for improving therapeutic targeting.
The main objectives of the project are:
(i) **To characterize canonical and non-canonical EZH2 activities**, focusing on variations in its interacting partners and post-translational modifications, using integrated approaches such as proteomics, transcriptomics, epigenomics, metabolomics, and gene editing.
(ii) **To determine the impact of these activities on leukemia development**, specifically in acute myeloid leukemia (AML).
(iii) **To evaluate the therapeutic potential of targeting EZH2 non-canonical functions** using preclinical models.
By combining complementary expertise, this project aims to identify the molecular pathways underlying the context-dependent functions of EZH2. Ultimately, these insights will contribute to improving diagnosis, prognosis, and treatment strategies for leukemia patients.

Main achievements within the first year of the IMSUT project

Analyses of functional mutations of EZH2: establishing the molecular tools

In this work, we aim to precisely evaluate the role of EZH2 phosphorylation and its contribution to the diverse functions of EZH2 during hematopoiesis and leukemogenesis. To achieve this, during the first year of the project, in collaboration with A. Iwama's laboratory we generated a series of phosphodead and phosphomimetic EZH2 mutants targeting residues S363, S367, S369, and T487.

These mutant forms will be compared to other mutants included S21D (known to turn off canonical EZH2 activity), F145A/F171A (mutation in the transactivation domain of EZH2), N673I (mutation occurring post gene-therapy in sickle disease); R690H (predominant mutation in AML patients with undetermined potential), Y646 (predominant mutation in B lymphoma associated with increase canonical activity of EZH2). Expression of all mutant forms has been validated in HEK293T cells. We are now introducing these constructs into EZH2-deficient cell lines generated by CRISPR/Cas9 (already established in the laboratories).

Given that the canonical activity of EZH2 depends on its interaction with core PRC2 components such as SUZ12 and EED, we hypothesize that disrupting these interactions may promote EZH2 non-canonical functions. We also generated SUZ12 CRISPR knockout AML cell lines, providing a complementary cellular model to specifically investigate non-canonical EZH2 activity.

Spin off: The characterization of EZH2 mutants and the development of dedicated cellular models may also provide valuable tools for the scientific community, enabling further investigation of both canonical and non-canonical EZH2 functions and supporting the development of targeted therapeutic strategies.

Targeting EZH2 in leukemia

We demonstrated that chemoresistant AML cell lines, particularly those resistant to AraC, are highly sensitive to EZH2 degradation, exhibiting approximately a 50-fold increase in sensitivity compared to AraC-sensitive cells. In contrast, these resistant cells do not respond to the catalytic EZH2 inhibitor Tazemetostat.

No synergistic effect was observed between EZH2 degradation and AraC treatment, indicating no added benefit of this combination therapy. However, a strong synergistic effect was identified between EZH2 degradation and retinoic acid (RA), a differentiation agent, leading to efficient elimination of AML cells in vitro. These findings suggest that EZH2 degradation may sensitize chemotherapy-resistant AML cells to RA.

Importantly, during this year, we showed that EZH2 deletion in CRISPR-based models phenocopies the effects observed with the EZH2 degrader in combination with RA, further supporting the specificity and relevance of this therapeutic strategy.

Expected Impact

This project will provide important insights into how post-translational modifications regulate the activity of a key epigenetic factor. By uncovering how EZH2 phosphorylation shapes its functional diversity, we expect to advance our understanding of both normal hematopoiesis and leukemia biology.

Importantly, this work has strong translational potential. By identifying mechanisms of therapy resistance and novel ways to target EZH2, it may contribute to the development of more effective therapeutic strategies for patients with acute leukemia, particularly in refractory or relapsed settings.

More broadly, this project will help establish a conceptual framework for understanding how dynamic regulation of epigenetic regulators influences disease progression and treatment response