METTL3 inhibitors screening cascade for new drug discovery

Kang Sun, Yuhong Chen, Dongmei Zhao, Haiting Dai, Yingxue Bai, Jiangtao Lin, Tao Li, Qiang Xia and Tiejun Bing
Department of Biochemistry, Center for In Vitro Biology, ICE Bioscience InC.

ICE Innovative CRO+Explorer ICE Bioscience

Introduction

Methyltransferase-like 3 (METTL3) is the only catalytic subunit of the transferase complex responsible for m6A modification, and it has been found to play a key role in tumorigenesis, tumor growth, metastasis and tumor drug resistance. In recent years, METTL3 has attracted more and more attention as a potential target for cancer treatment.

On this basis, we established an in vitro and in vivo screening platform on METTL3/14 complex, which can realize high-throughput screening of METTL3 inhibitors in vitro experiments. High purity METTL3/14 protein complex has been successfully purified and used in develop METTL3/14 biochemical experiments. We have also established different cellular assays, including proliferation and m6A RNA Methylation quantification assay. Lastly, CDX models utilizing different Ovarian cell lines have been validated. We aim to aid the discovery of new drugs through the METTL3/14 screening cascade.

ICE Innovative CRO+Explorer 爱思益普生物

ICE Information Retrieval System

本数据库包含47689条专利数据 提交

Patent Number	Owner Company	Target	Biochemical	Cellular
WO-2022074379	Storm Therapeutics Ltd	METTL3	YES	YES
WO-2022074391	Storm Therapeutics Ltd	METTL3	YES	YES
WO-2022081739	Accent Therapeutics, Inc	METTL3	YES	YES
WO-2023104209	Shanghai OnCusp Therapeutics Ltd	METTL3	YES	YES
WO-2023129933	858 Therapeutics Inc.	METTL3	YES	YES
WO-2023151697	Sichuan Haisco Pharmaceutical Co Ltd	METTL3	YES	YES
WO-2024056099	Global Health Drug Discovery Institute	METTL3	YES	YES
WO-2024145483	HTG Molecular Diagnostics Inc	METTL3	YES	YES

Figure1. Search results for METTL3 in ICE Information Retrieval System.

METTL3/14 Recombinant Proteins

Recombinant human METTL3 protein with N-terminal Flag tag was co-expressed with recombinant human METTL14 with N-terminal 6x His tag. Recombinant human METTL3/METTL14 protein was purified by Ni-NTA affinity and followed by SEC chromatography. For more information, please visit ICE Protein Platform: https://enpro.ice-biosci.com/

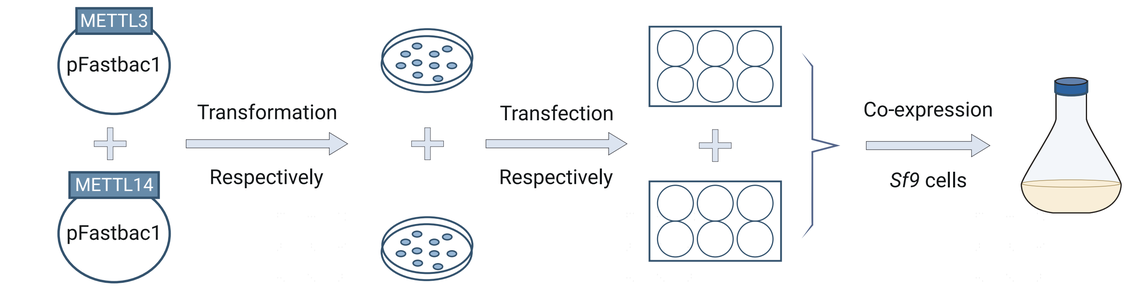
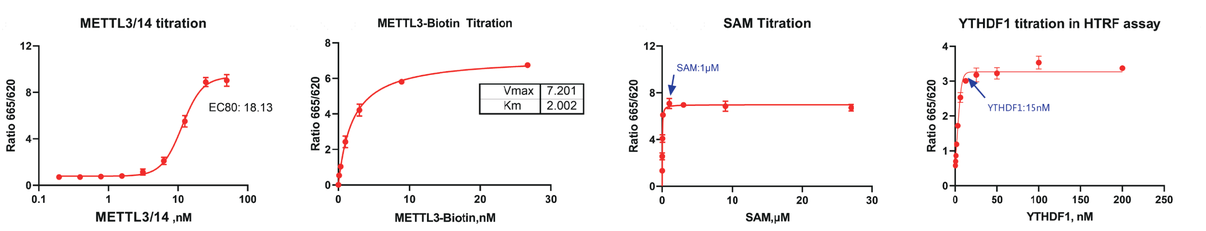


Figure2. A. Procedure for METTL3/14 protein complex expression. B. SDS-PAGE and HPLC for METTL3/14 protein complex purification.



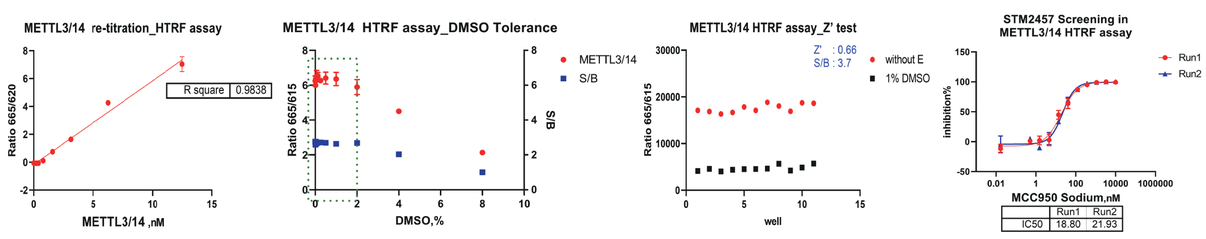


Figure3. Procedure of METTL3/14 protein complex HTRF assay Validation. The assay development cycle is usually one week.

METTL3/14 Activity Assay

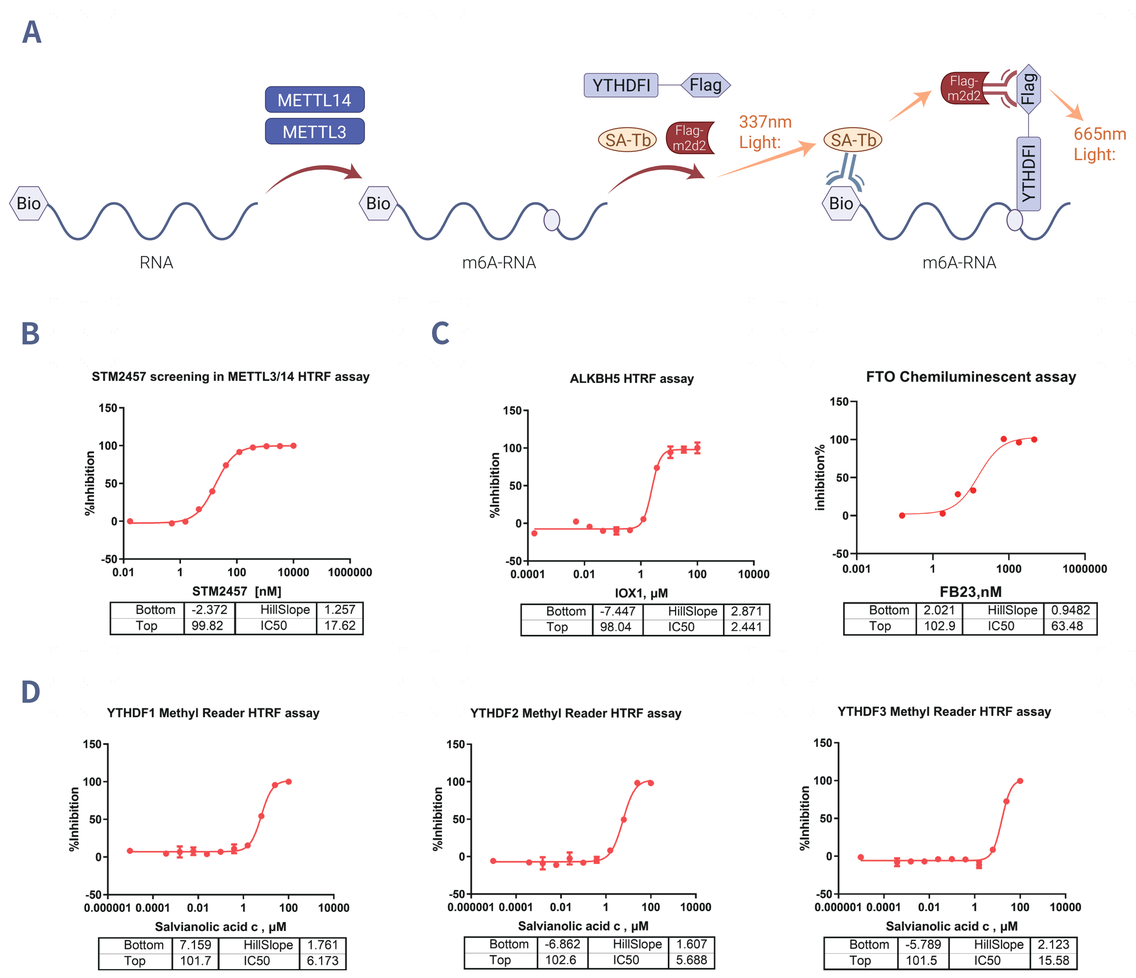


Figure4. A. Model diagram of METTL3/14 TR-FRET assay. B. The results of commercialized inhibitors in METTL3/14 biochemical assays. C. The results of reference in m6A "eraser" biochemistry assay. D. The results of reference in m6A "reader" biochemistry assay.

METTL3/14 Cellular Assays

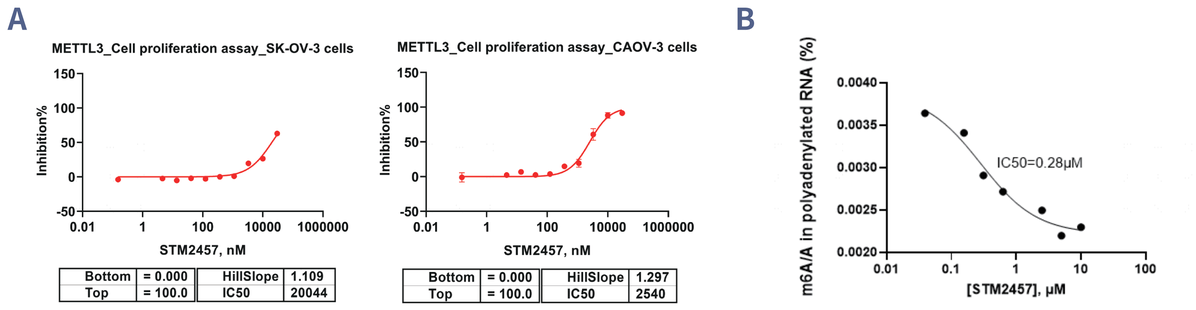


Figure5. A. The results of commercialized inhibitors in Cell proliferation assay(SK-OV-3/CAOV-3). B. The results of commercialized inhibitors in Cell m6A RNA Methylation quantification assay.

Animal Modeling

CDX modeling utilizing different cell lines has been established for the efficacy study as shown below to expand the drug discovery cascade to the in vivo experiments.

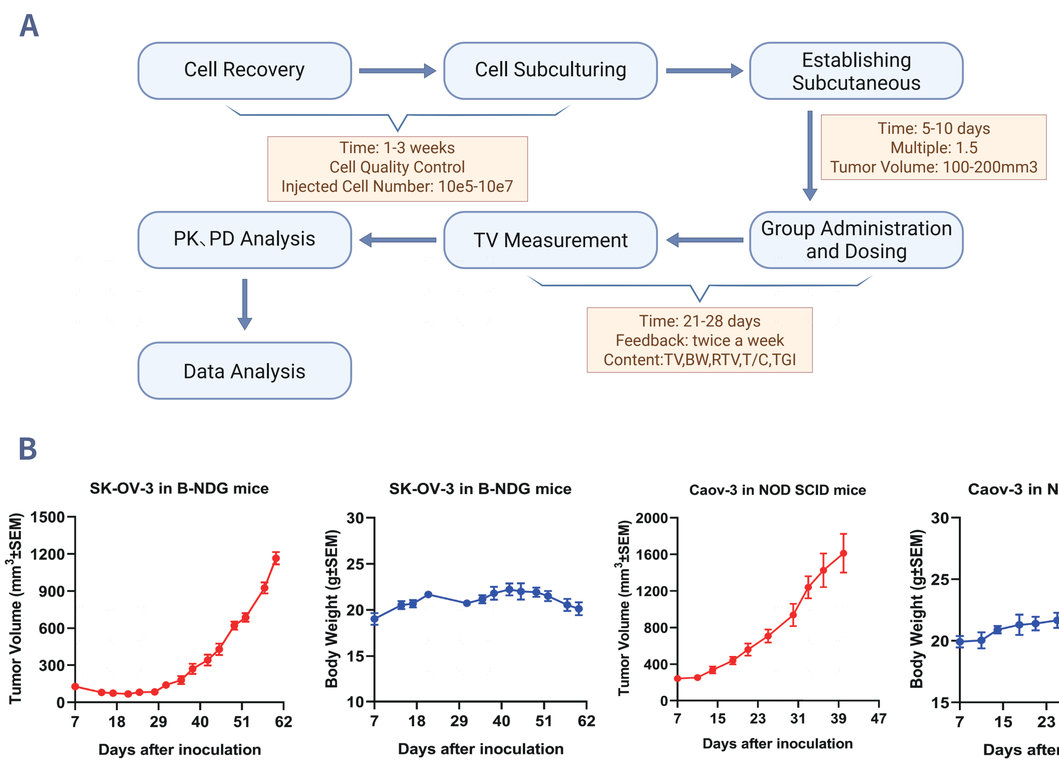


Figure6. A. Procedure for animal tumor model set up and PK/PD analysis. B. The results of Ovarian adenocarcinoma cell line CDX model and animal body weight.

Conclusions

METTL3 is a crucial hub in tumor growth and progression, regulating the splicing, stability, and expression of a wide range of genes through m6A modifications. Drug development targeting METTL3 has great potential, as METTL3 is highly expressed in a wide range of tumors. We constructed an experimental cascade from in vitro to in vivo, which is composed of protein production, biochemical assays, cellular assays, and animal modeling. We aim to aid the discovery of new drugs through the METTL3/14 screening cascade.

Acknowledgements

We thank ICE Bioscience for providing the research fund of establishing CDK screening cascade. We thank Dr. Li Yan and Dr. Yingji Li for their personal support throughout this research.