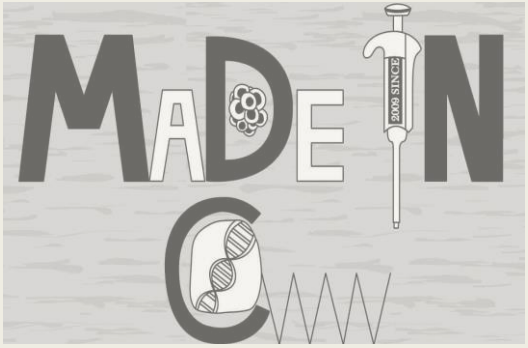
Mechanisms of the inhibitory effect of hinokitiol in repressing EZH2 expression in breast cancer cells



Speaker：董俊仁
Supervisor：張文瑋 教授
Date：09.12.2018

Introduction

Breast Cancer

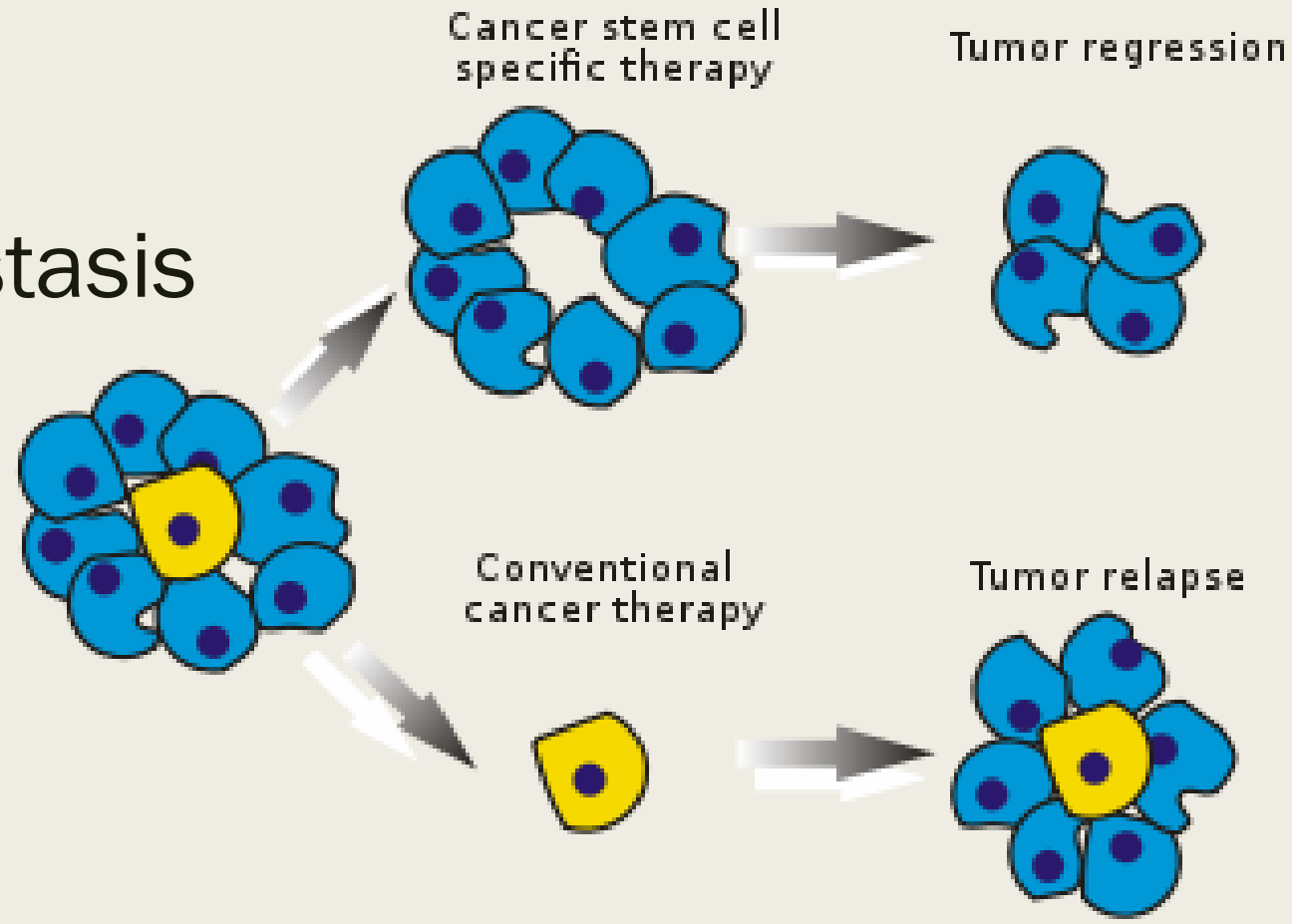
- Over 10 thousand people are diagnosed
- 5-year overall survival rate is 85%
- 10~40% of recurrence

Cancer Stem Cells (CSCs)

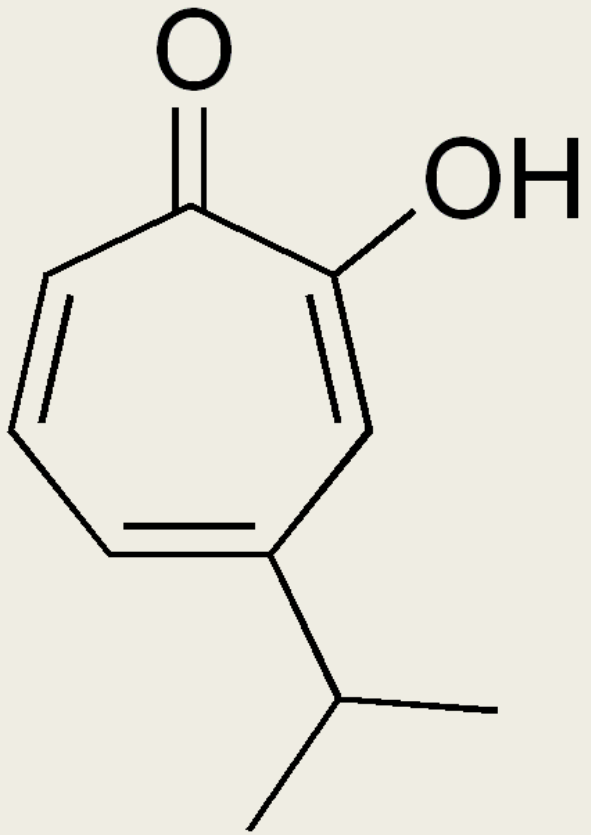
- Self-renewal
- Differentiation
- Tumor initiation, metastasis

  , recurrence

(Neethan A.L. et al., Annu. Rev. Cell Dev. Biol, 2007)



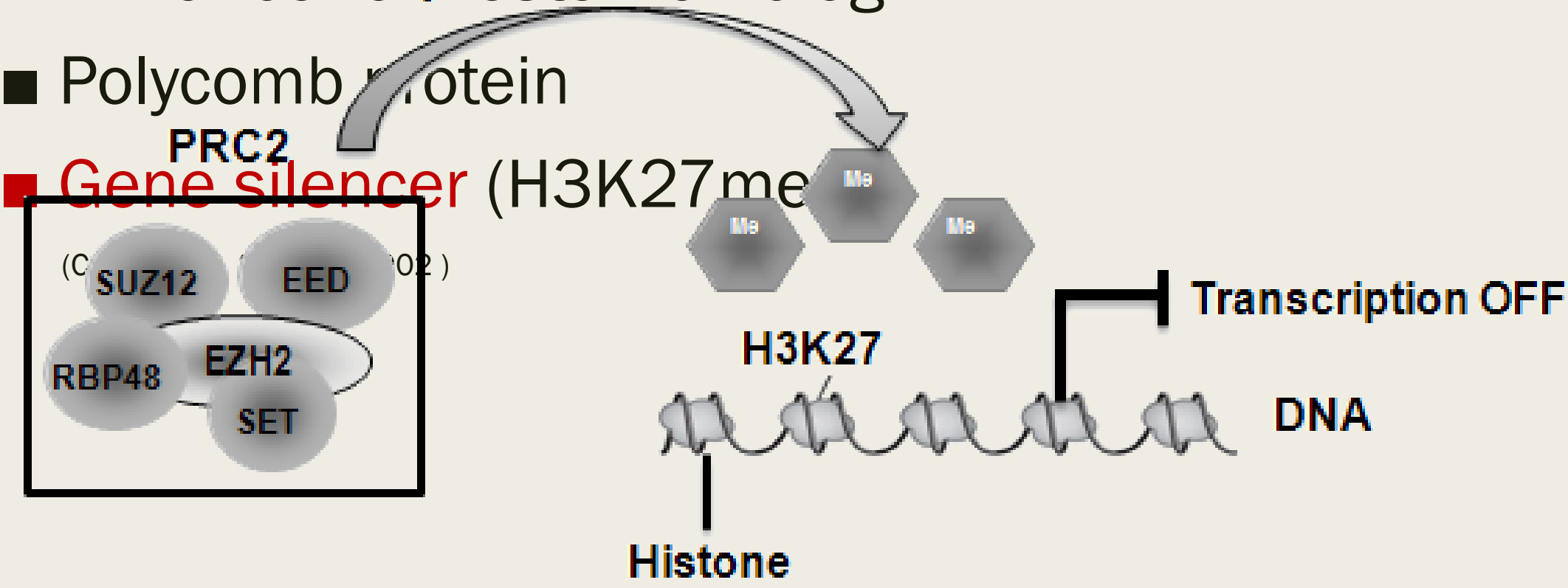
Hinokitiol (檜木醇)



- A tropolone-related compound
- Isolated from Taiwanese hinoki
- Anti-microbe (Shih YH et al., PloS one, 2014)
- Anti-inflammation (Morita Y, et al., Biocontrol Sci, 2007)
- Anti-tumor capacity (Liu S, et al., Cancer letters, 2009)

EZH2

- Enhancer of Zeste Homolog 2
- Polycomb protein
- Gene silencer (H3K27me



EZH2

- Highly-expressed in gastric cancer

  (Pan YM et al., Mol Cancer, 2016)

- Promotes liver cancer progression

  (Chen S et al., J Exp Clin Cancer Res, 2018)

- Epigenetic silencing of the tumor suppressor gene in prostate cancer (Kunderfranco P et al., PLoS One, 2010)

EZH2

- Up-regulation in ovarian cancer cells

  (Garipov A et al., Mol Cancer Res. 2013)

- Down-regulation tumor suppressor miR in glioblastoma (Michiel S et al., Oncotarget. 2010)

- EZH2 is a marker of aggressive cancer

micro RNA (miR)

- small non-coding RNAs with 19–23 nucleotides
- binding to the 3′-UTR to cause translation inhibition or mRNA degradation

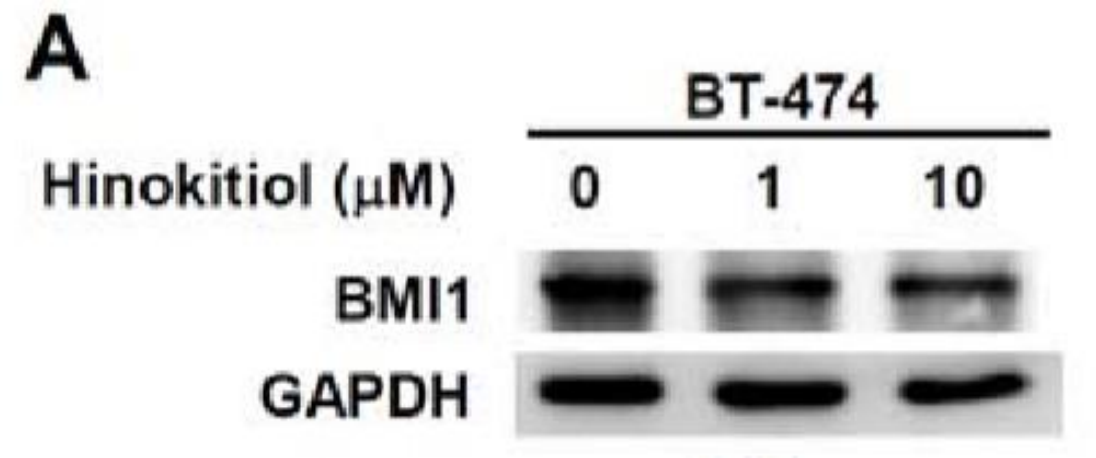
micro RNA (miR)

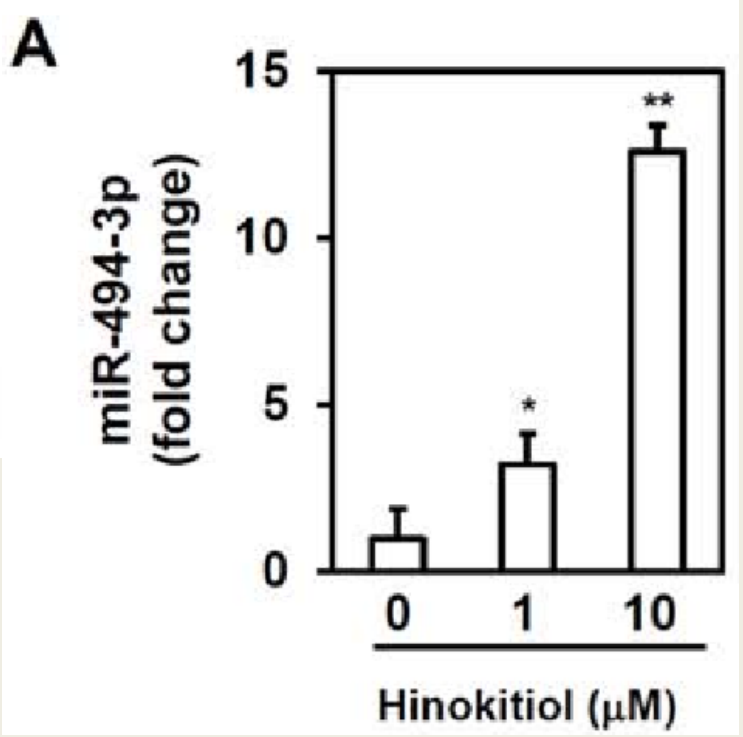
- miR-145-5p acts as a tumor suppressor in head and neck cancer (Yamada Y et al., Int J Oncol. 2018)
- miR-494-3p inhibits cell proliferation and metastasis in osteosarcoma (Yuan W et al., Mol Med Rep. 2017)
- EZH2 can suppress other tumor-suppressor miR in liver cancer (Chen S et al., J Exp Clin Cancer Res. 2018)

Motivation

Previous study of our lab

- Hinokitiol inhibits BMI1 protein expression
- And up-regulates miR-494-3p





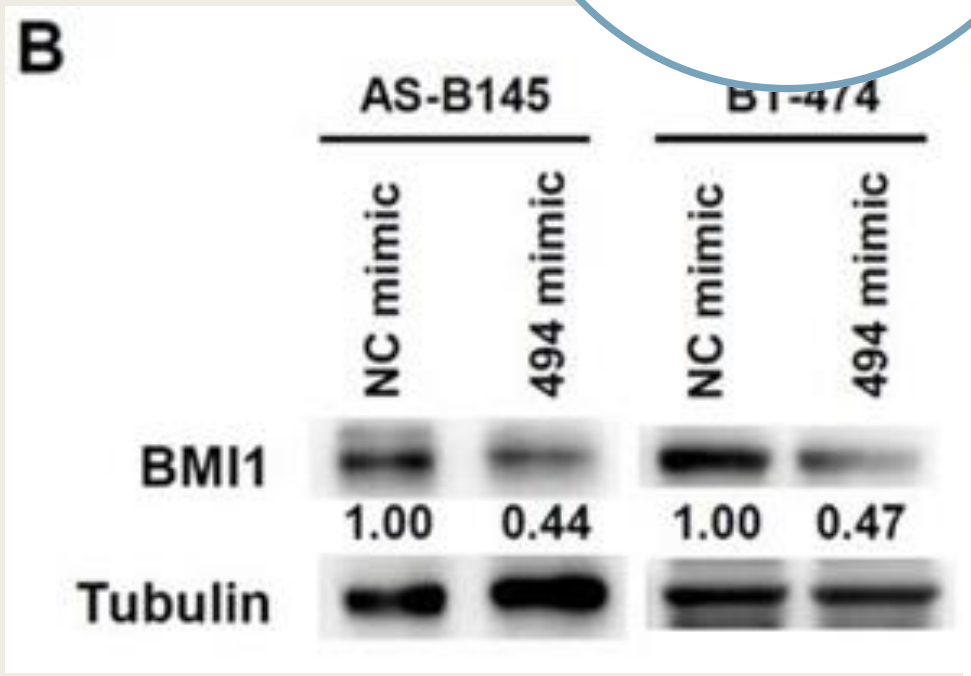
(SM Chen et al., Oncotarget. 2017)

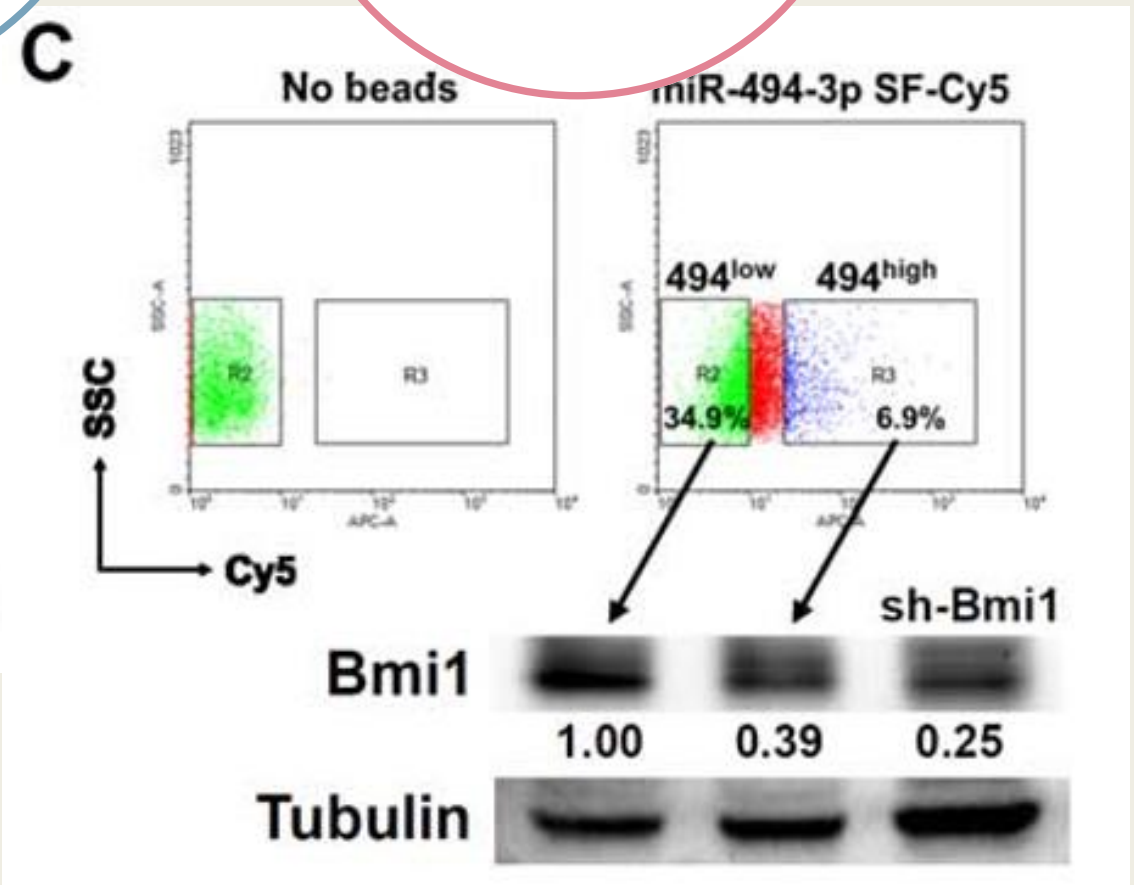
Previous s our l

- miR-494 inh otein

High miR-494-3p expression

Low miR-494-3p expression





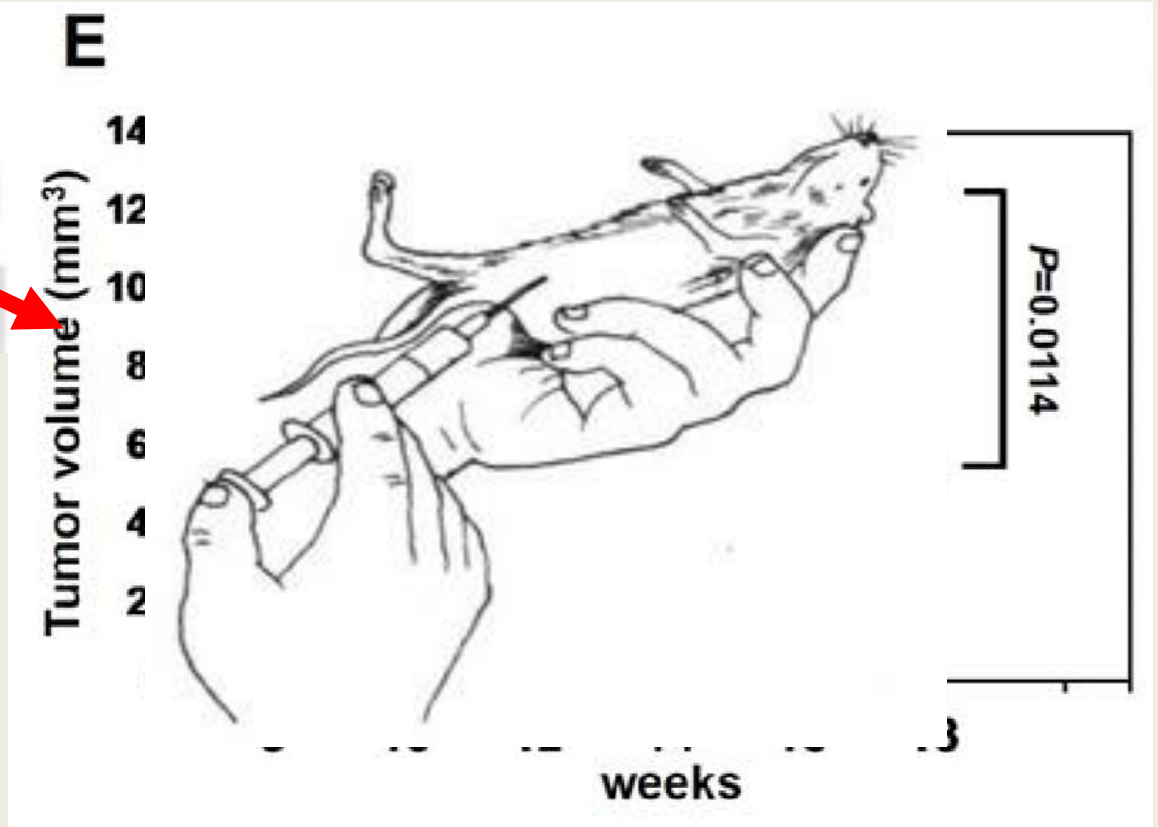
(SM Chen et al., Oncotarget. 2017)

Previous study of c

- The [illegible] of miR494-3p overexpressed BT-474 [illegible] ificantly slower

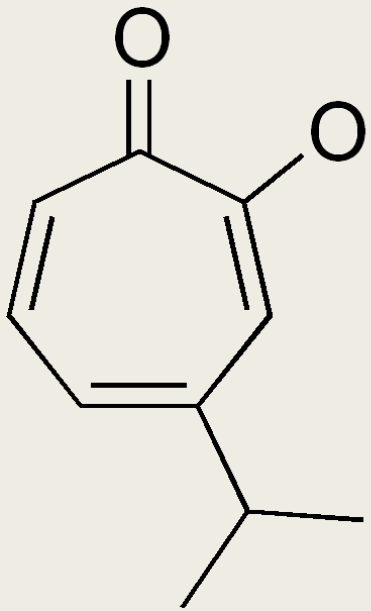
BT-474 cells

miR-494-3p



(SM Chen et al., Oncotarget. 2017)

hinokitiol



miR-494-3p

BMI1

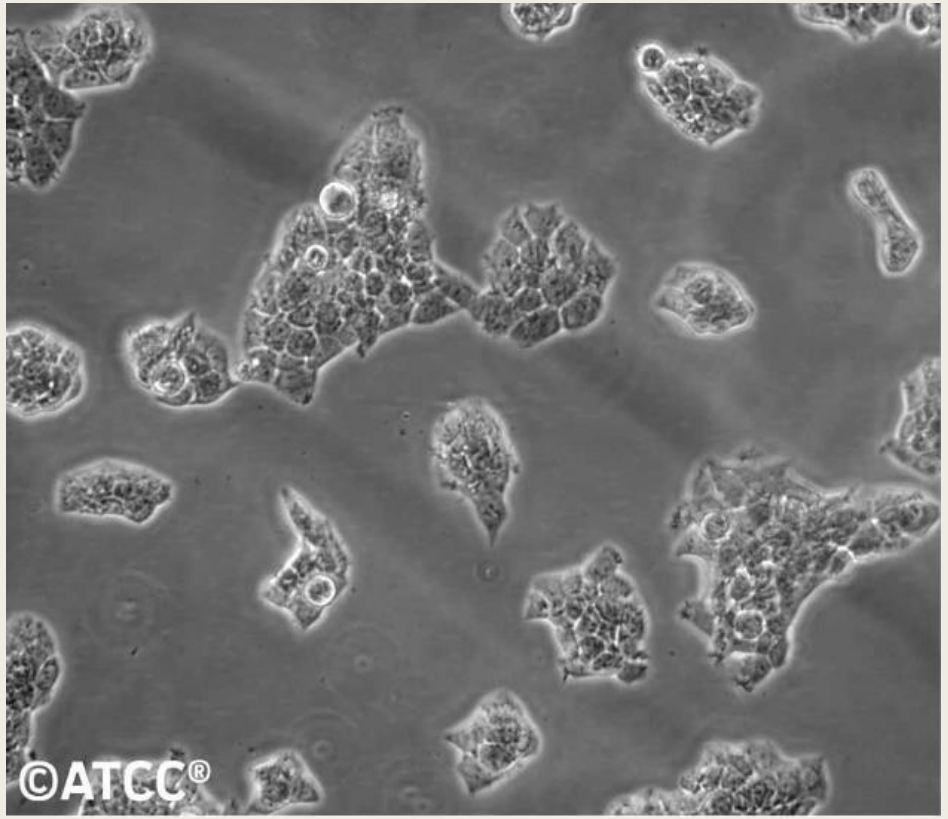
CSCs self-renweal

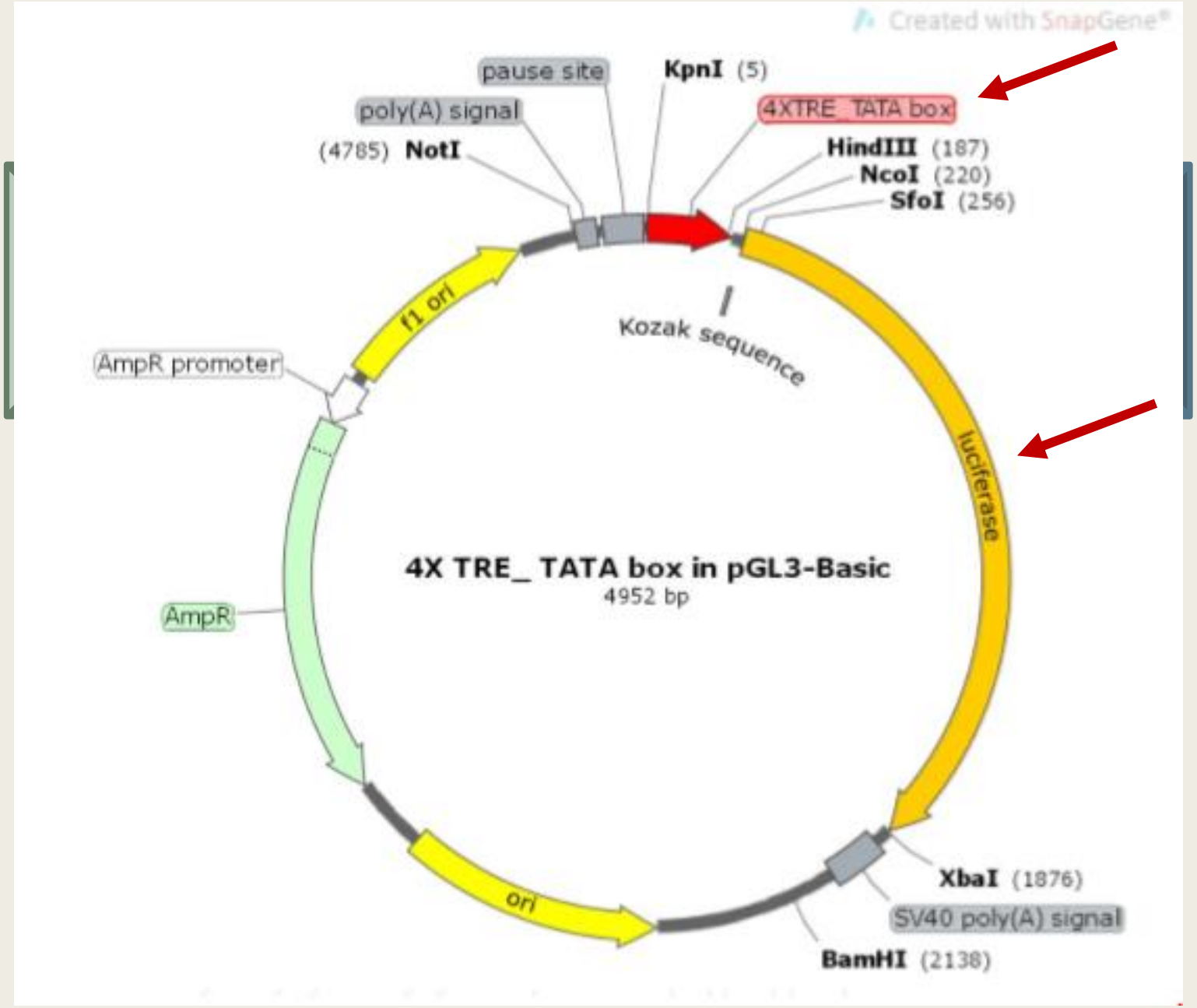
Materials & Methods

Cell line & Treatment

- BT-474 human breast carcinoma cell line

- Treated with hinokitiol for 48 hours in different concentration

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KpnI (5)
poly(A) signal
4XTRE_TATA box
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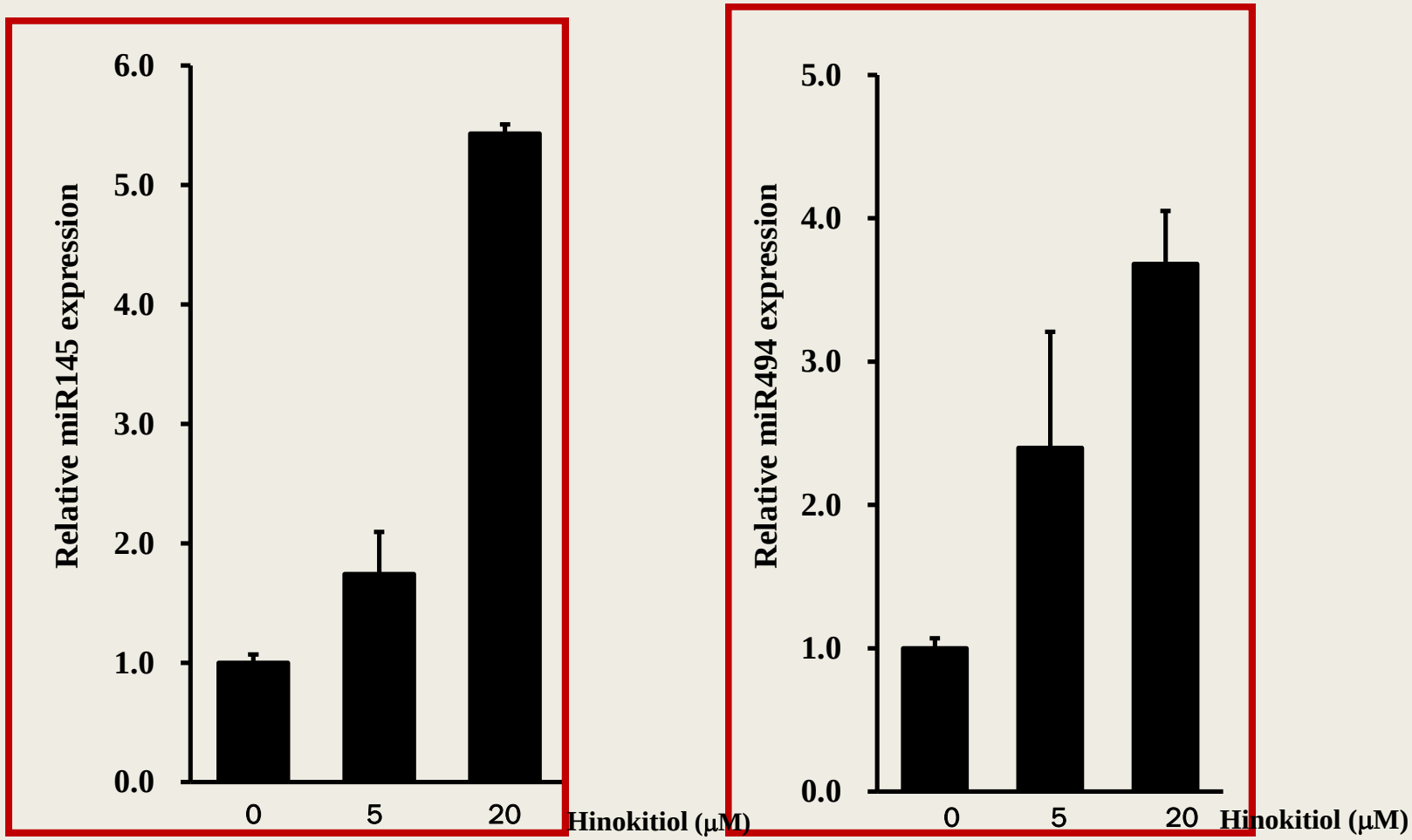
HindIII (187)
NcoI (220)
SfoI (256)
f1 ori
Kozak sequence
AmpR promoter
luciferase
4X TRE_ TATA box in pGL3-Basic
4952 bp
AmpR
ori
XbaI (1876)
SV40 poly(A) signal
BamHI (2138)

Result

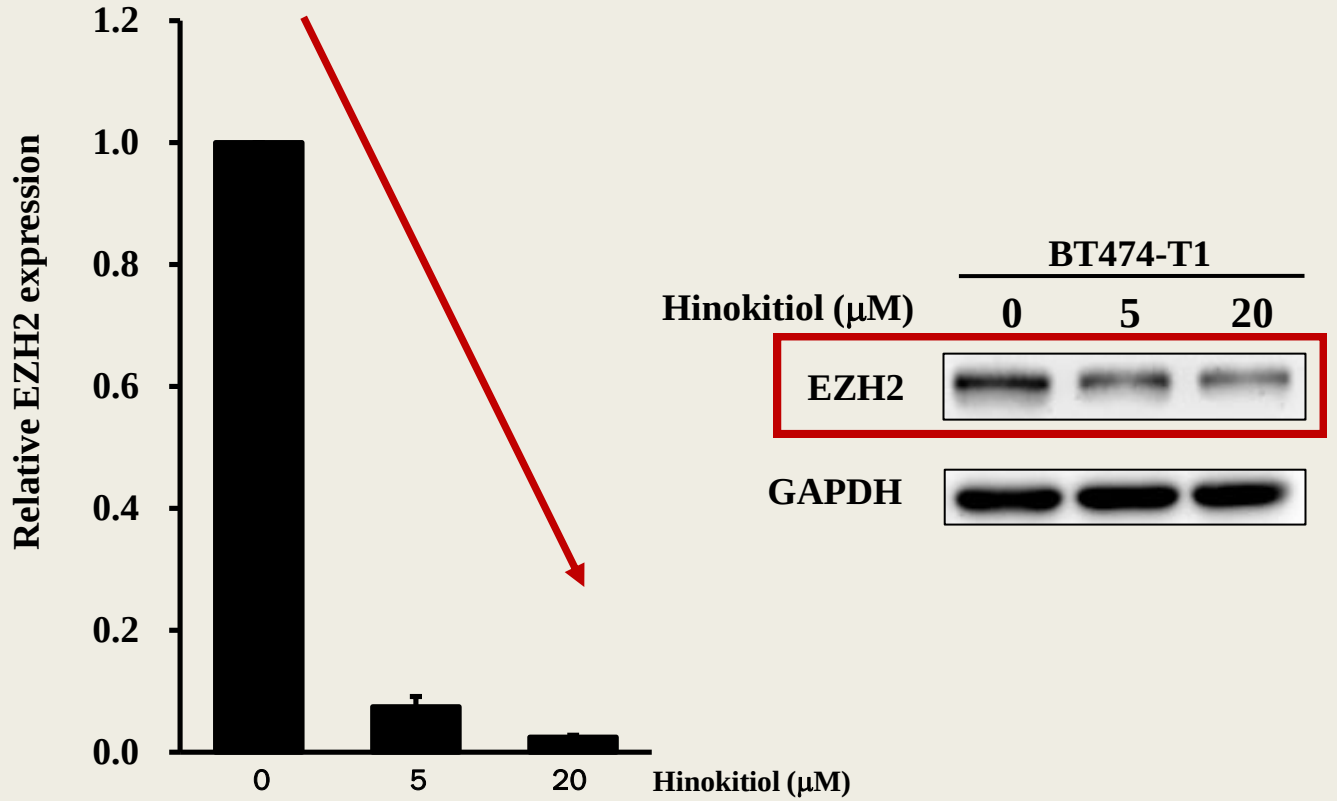
Hinokitiol up-regulates miRs

- miR-145-5p(A) and miR-494-3p(B) both up-regulated after hinokitiol treatment



EZH2 expression being suppressed

- EZH2 expression is suppressed both in RNA and protein level
- Transcriptional inhibition



Transcription Factors

which are indicated to regulate EZH2 in different pathway

- SIRT1 (sirtuin 1)

  (Lu L et al., Chin Med Sci J. 2011)

- STAT3 (Signal transducer and activator of transcription 3)

  (Pan YM et al., Mol Cancer. 2016)

- Erg1/2/3 (ETS transcription factors)

  (Kunderfranco P et al., PLoS One. 2010)

- HIF-1α (hypoxia-inducible factor 1- alpha)

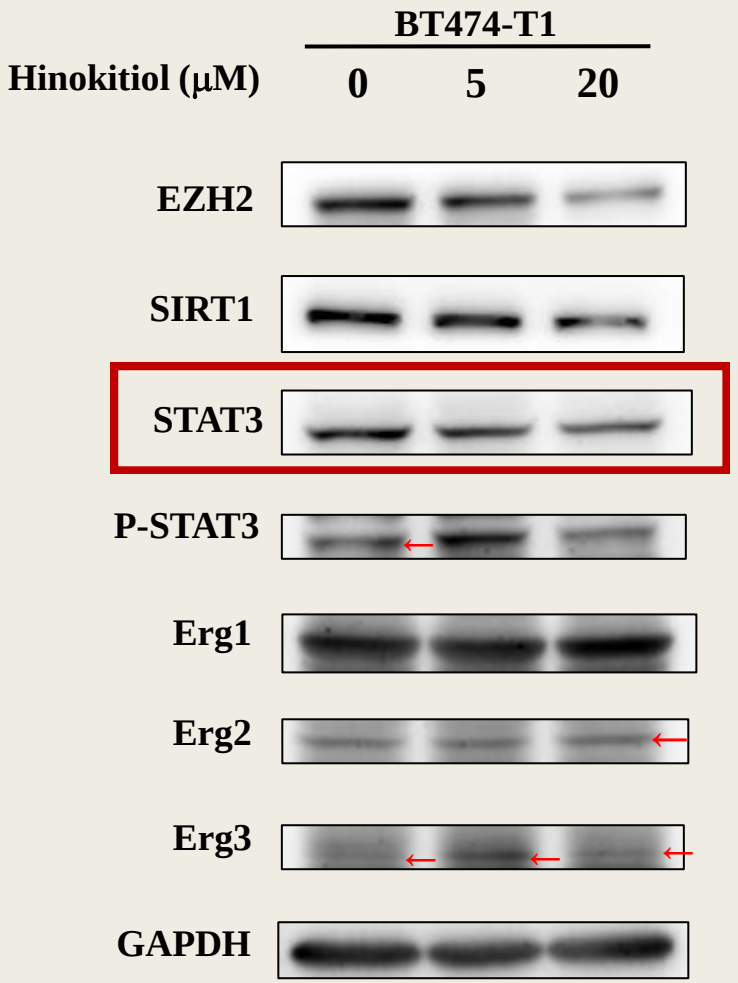
  (Pang B et al., Oncotarget. 2016)

- NF-YA (Nuclear transcription factor Y subunit alpha)

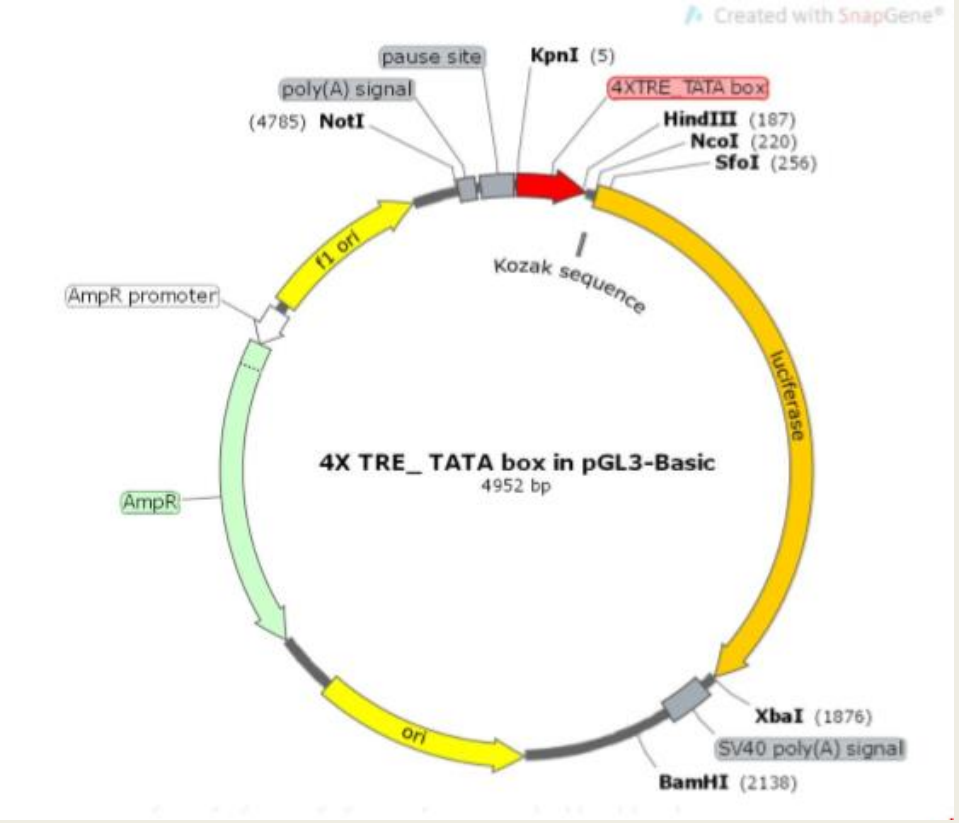
  (Garipov A et al., Mol Cancer Res. 2013)

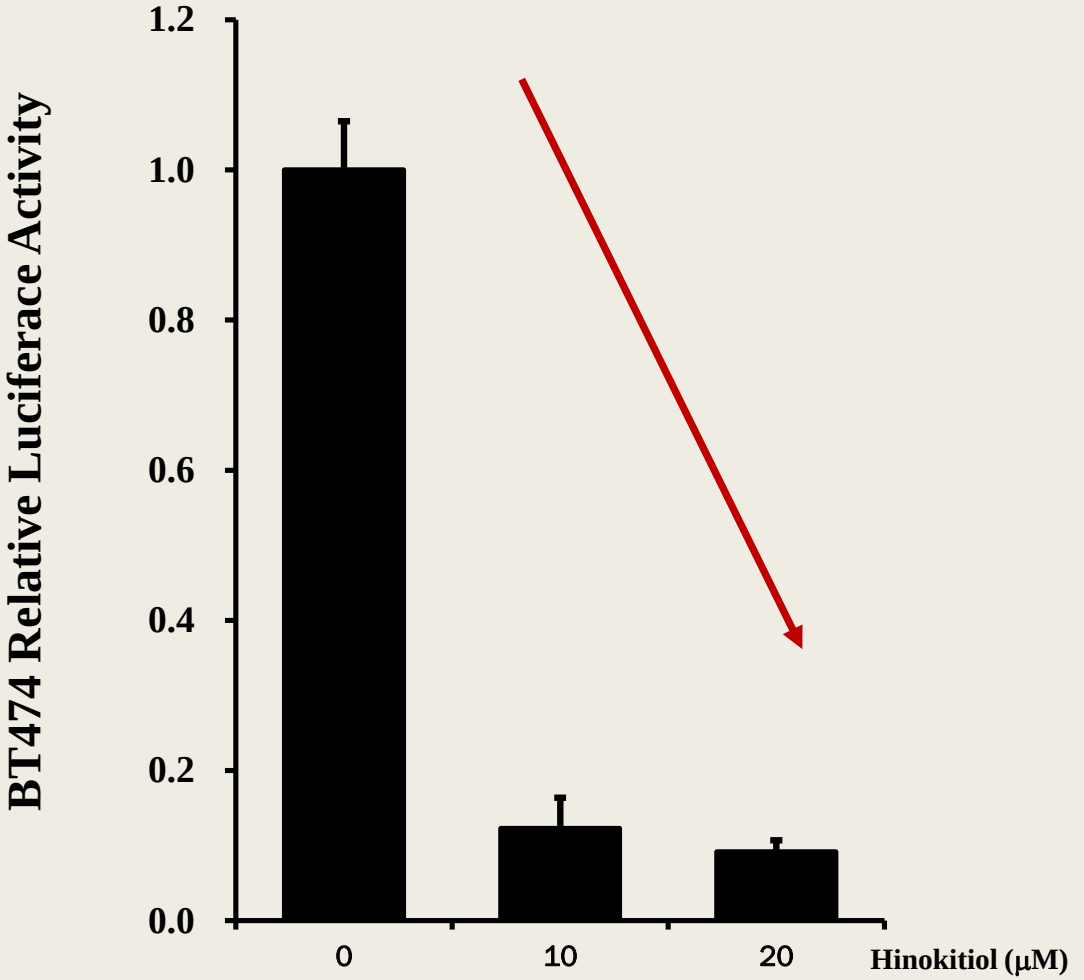
STAT3 down-regulated

- We assumed that STAT3 is related to the inhibitory effect of hinokitiol in EZH2 suppression

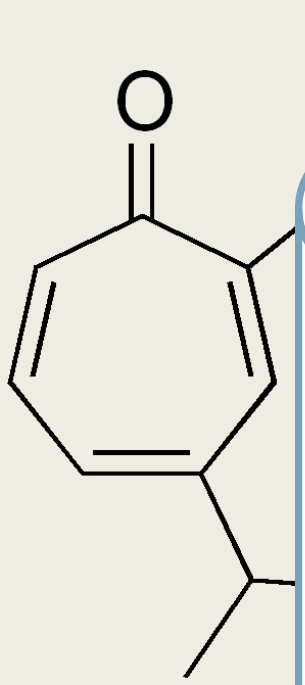


Decreased STAT3 activity





Summary



hinokitiol

Suppression of self-renewal and differentiation capacity of cancer stem cells

EZH2

miR-494-3p
miR-145-5p

Future Work

- Investigate the molecular mechanisms between EZH2 and miR-494-3p & miR-145-5p

- Further confirm whether STAT3 can affect the expression of EZH2, through knockdown or overexpression STAT3 in BT-474 cells

Thanks for Your Attention