Melatonin in the treatment of metastatic lung cancer : preclinical and molecular mechanism study

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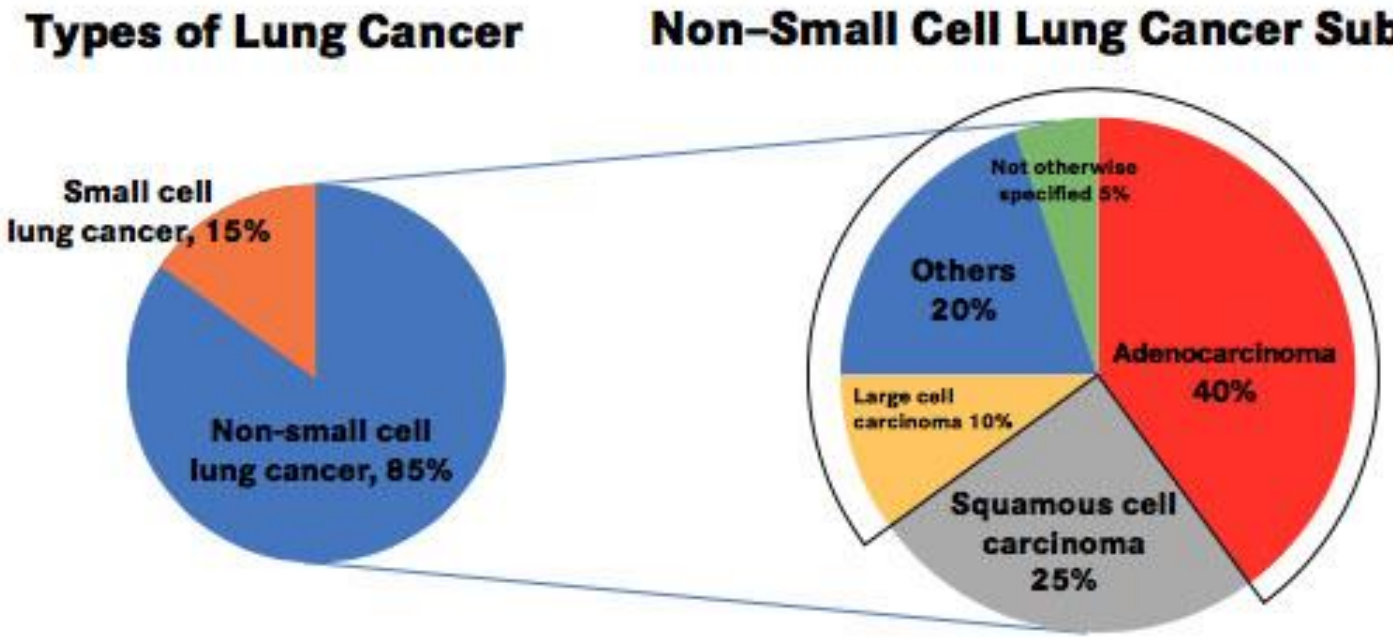
TSPCCM 2019 Congress, 2019/12/07

Types of Lung Cancer

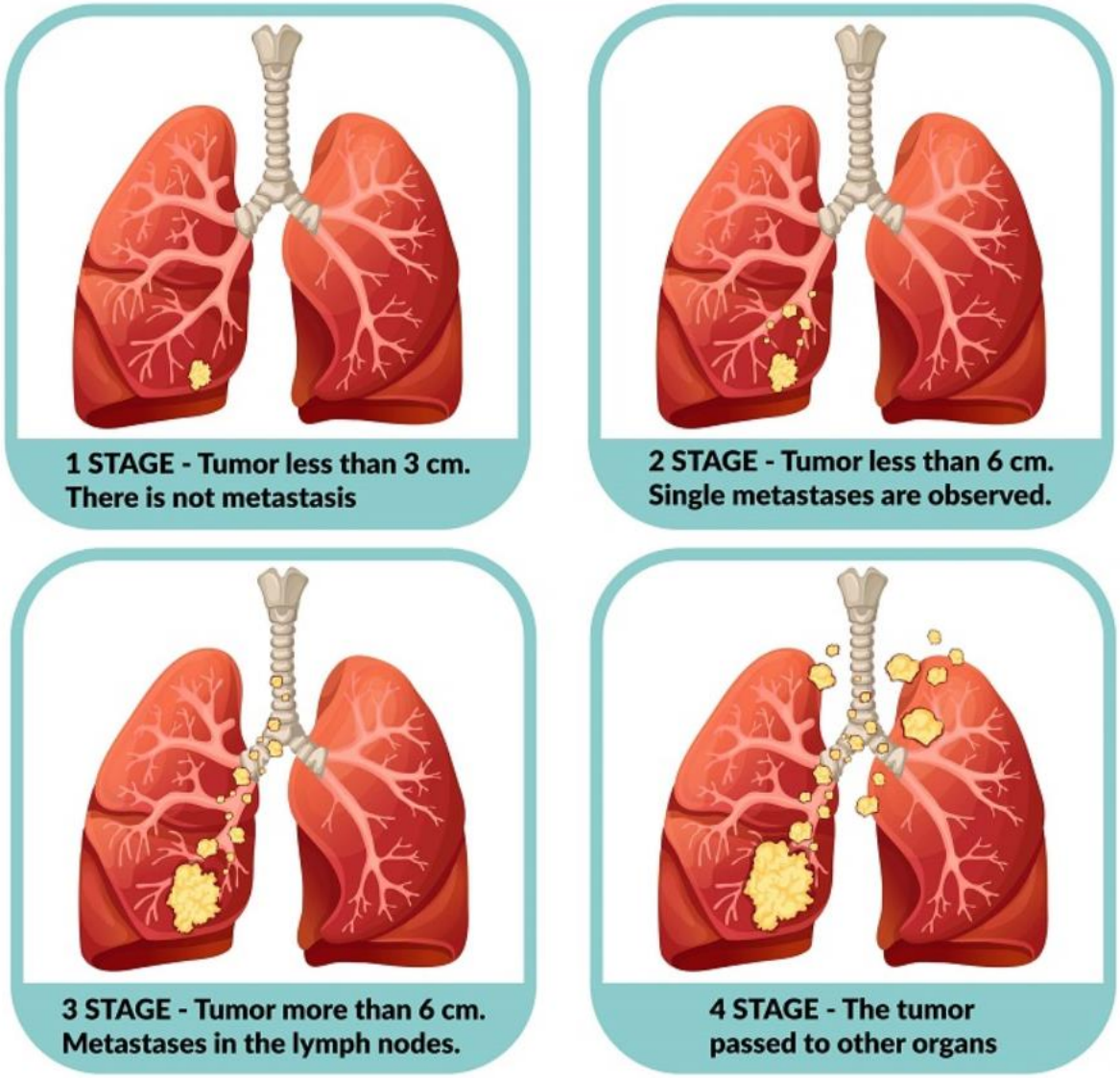
FIGURE 1. Types of Lung Cancer by Histology[1,4,5]

Types of Lung Cancer	
Non–small cell lung cancer	85%
Small cell lung cancer	15%
Non–Small Cell Lung Cancer Subtypes	
Adenocarcinoma	40%
Squamous cell carcinoma	25%
Large cell carcinoma	25%
Others	20%
Not otherwise specified	5%



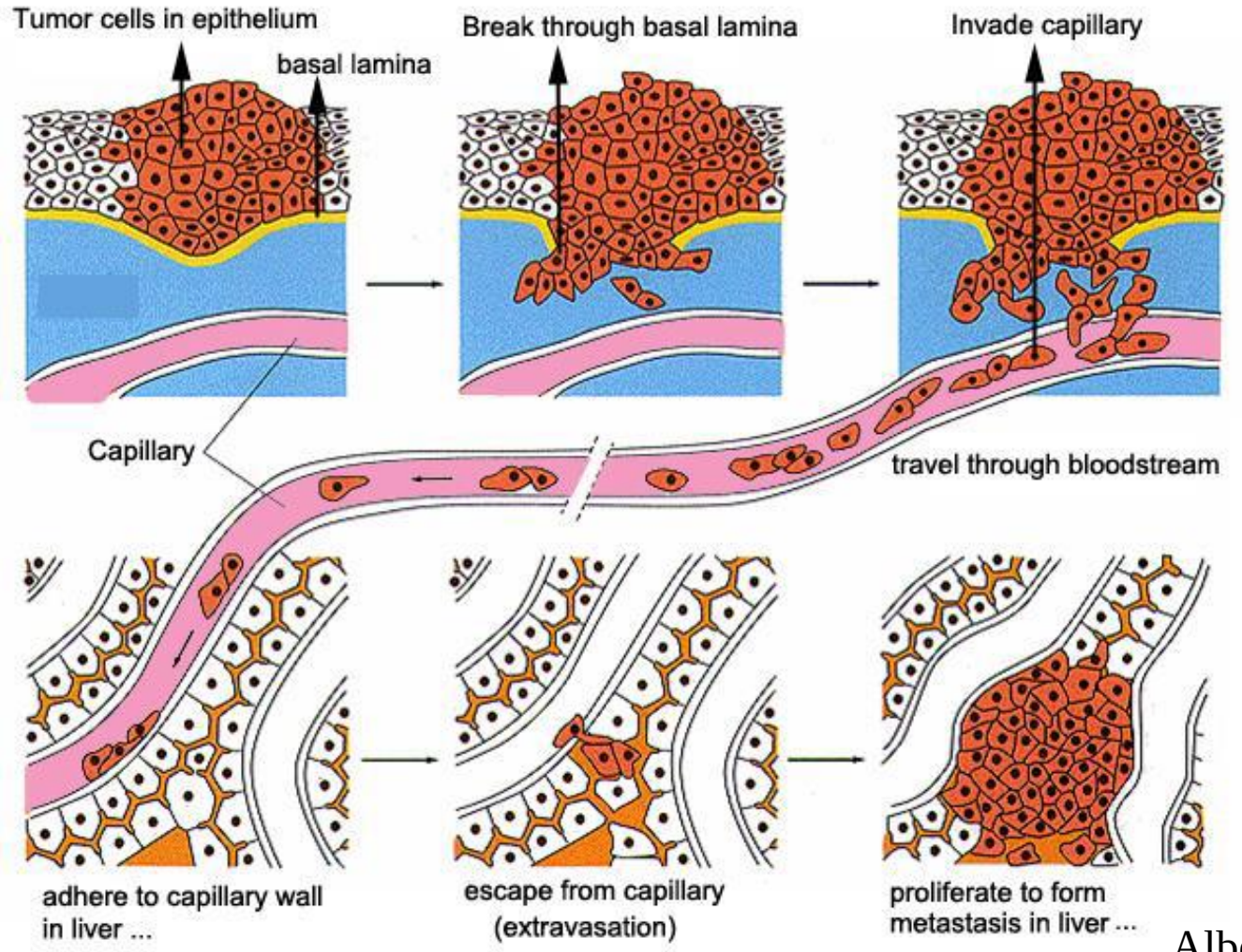
Nonbiomarker, Nonsquamous NSCLC: Diagnosis, Statistics, Staging, and Testing, 2017

Stages of Lung Cancer



Narayana Health

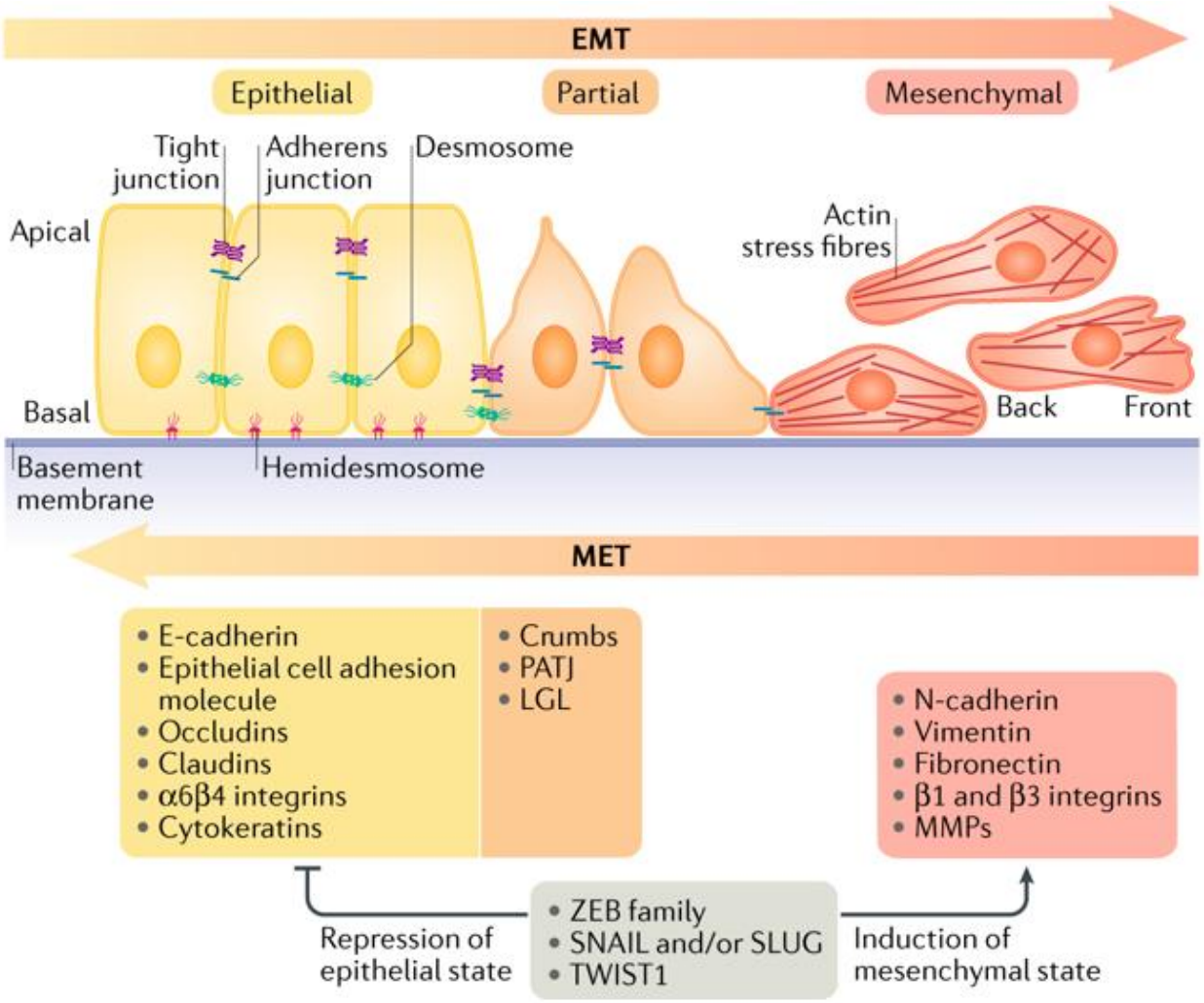
Metastasis in cancer



Schematic diagram of cancer cells hematogenous metastasis

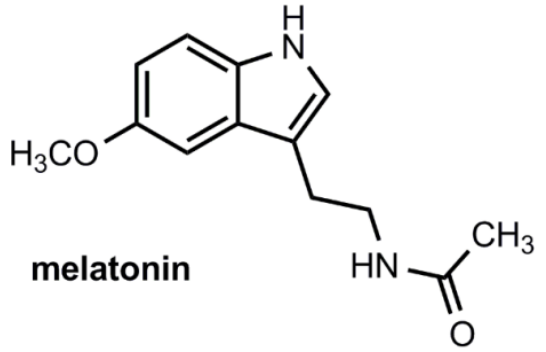
Alberts, B. (Bruce Alberts) et al.,2002

Epithelial Mesenchymal Transition, EMT



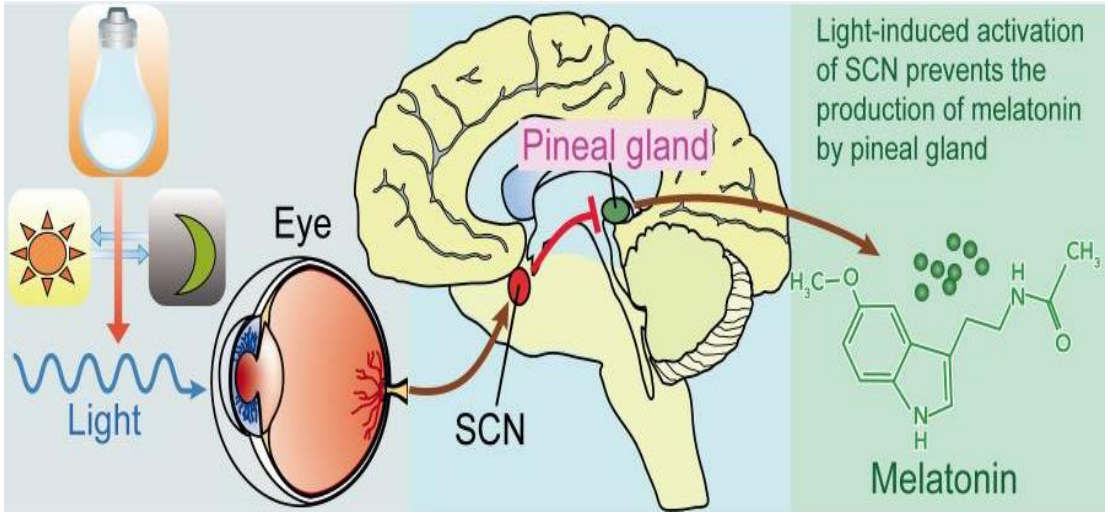
Anushka Dongre, 2018

Melatonin



melatonin

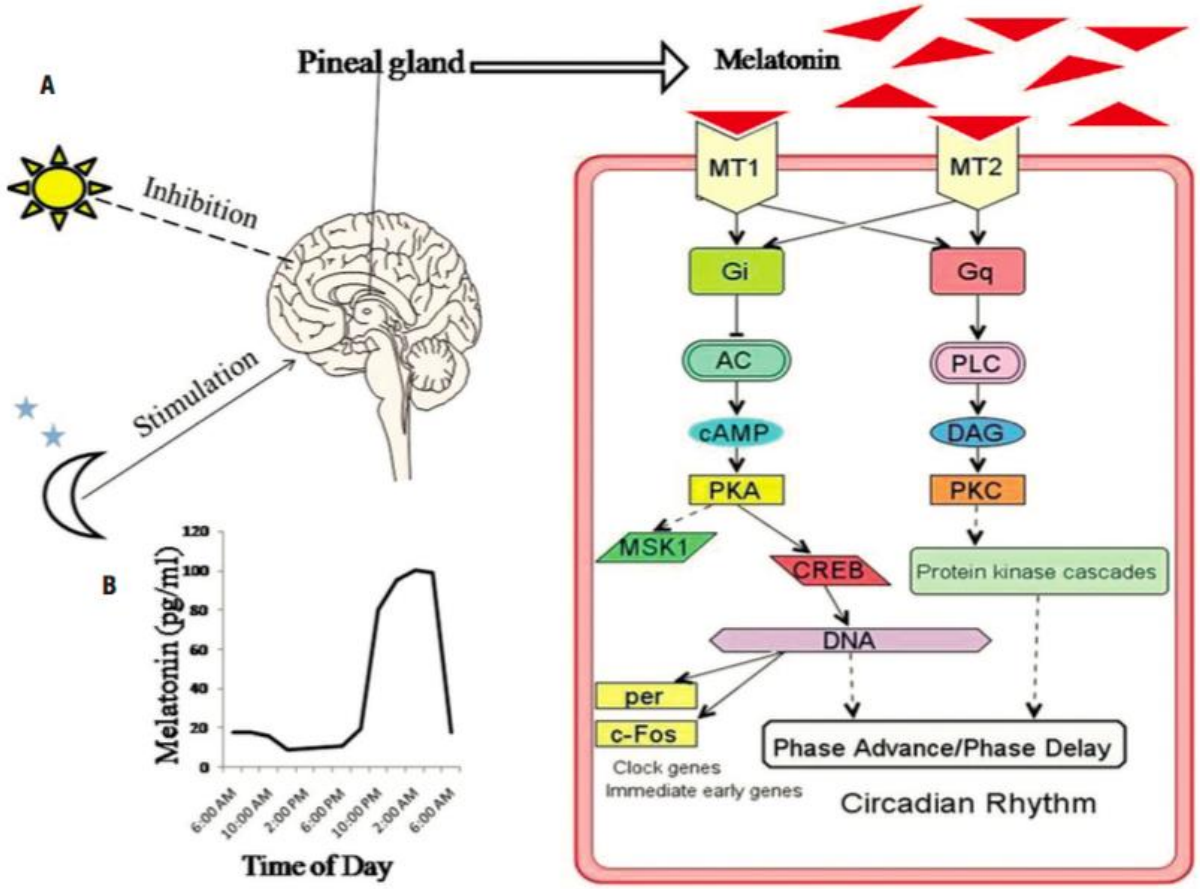
- **Melatonin : N-acetyl-5-methoxytryptamine**
- **Melatonin was only first discovered in a bovine pineal gland in 1958 by a dermatologist named Aaron Lerner.**
- **Melatonin, a hormone secreted from the pineal gland at night, plays roles in regulating sleep-wake cycle, pubertal development and seasonal adaptation.**
- **The concentration in plasma during night was found to be (80–100 pg/mL) and low levels during the day (10–20 pg/mL).**



Ma, Z., et al., 2016

Melatonin

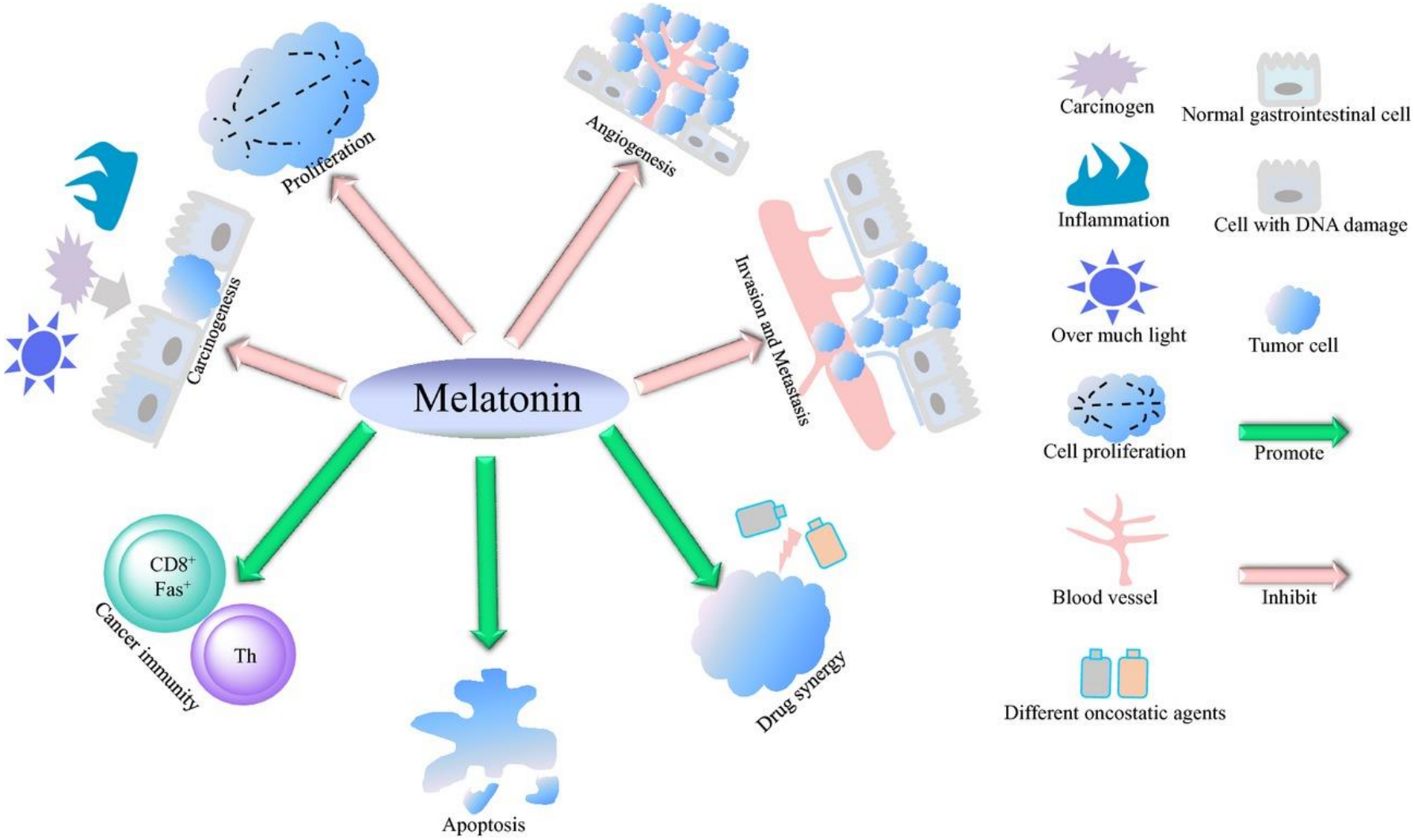
- **Melatonin affects by four mechanisms:**



1) **Binding to melatonin receptors in plasma membrane**
2) **Binding to intracellular proteins such as calmodulin**
3) **Binding to Orphan nuclear receptors**
4) **Antioxidant effect.**

Sharma, Shweta, et al, 2015

Mechanisms cancer of melatonin



Xin, Zhenlong, et al, 2015

Discussion

- ✓ **The possibility to improve the efficacy of chemotherapy in terms of survival by a concomitant administration of melatonin.**

J Pineal Res. 2003 Aug;35(1):12-5.

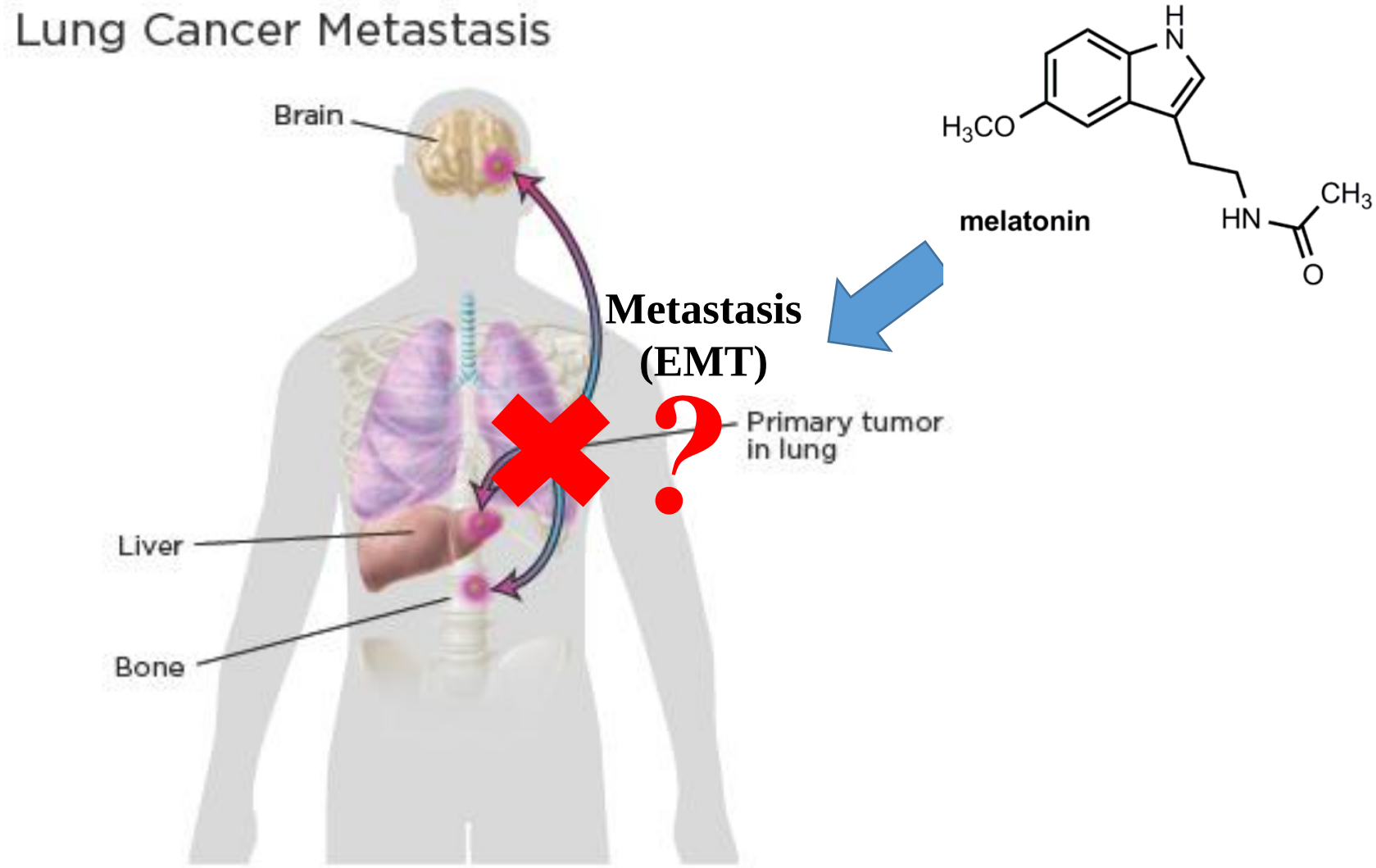
- ✓ **The literature also indicates that in the third and fourth stages, melatonin in lung cancer is lower than in normal body.**

Med Sci Monit. 2005 Jun;11(6):CR284-288. Epub 2005 May 25.

- ✓ **Melatonin has low toxicity and highly favorable compatibility, so treatment enhances the efficacy and reduces the side-effects of radio- or chemotherapies.**

Oncotarget. 2016 Jul 19;7(29):46768-46784. doi: 10.18632/oncotarget.8776.

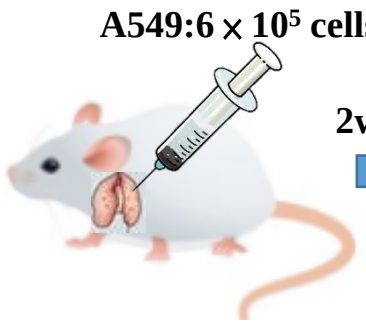
- Whether melatonin inhibits lung cancer metastasis by regulating EMT signalling pathway?



Whether melatonin inhibits the tumor metastasis in vivo?

Result

➢Whether melatonin inhibits the tumor metastasis *in vivo*?



A549:6 × 10^5 cells

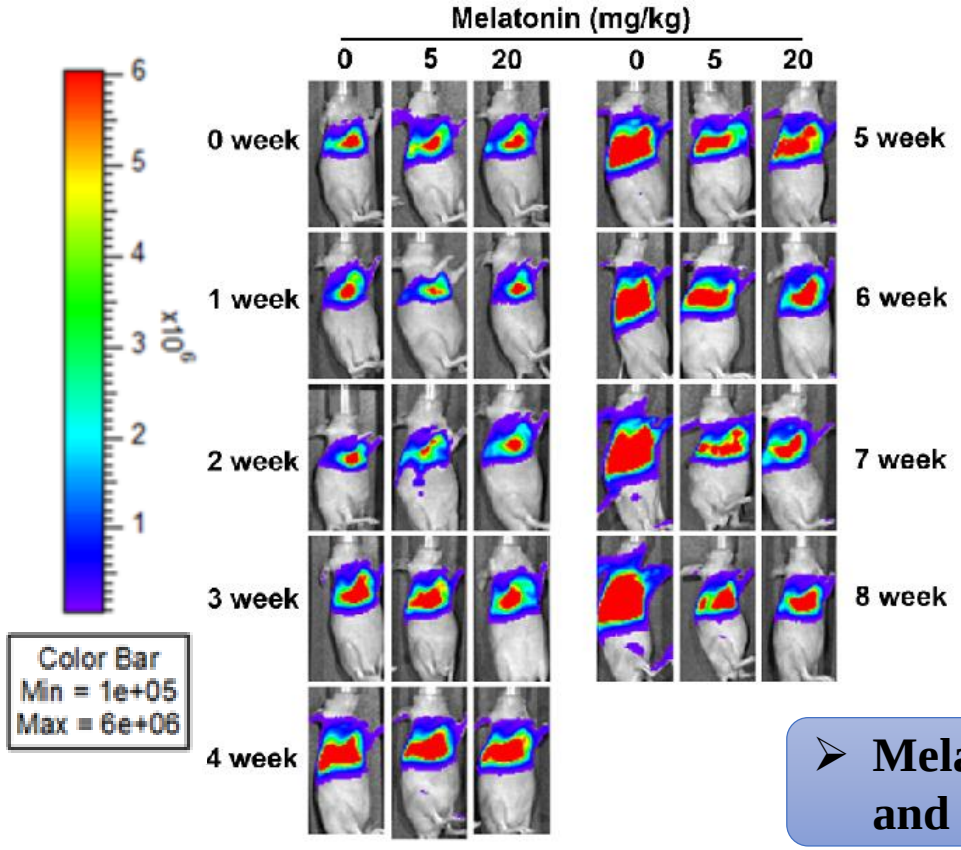
2weeks

Three times a week

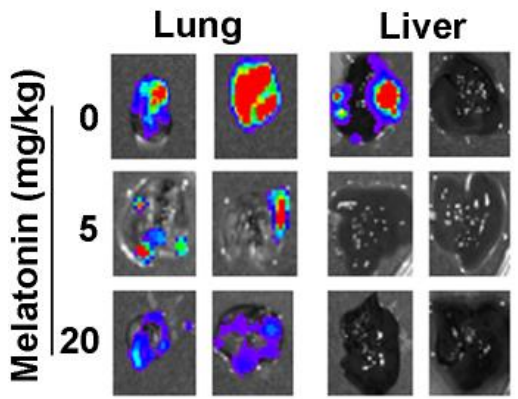
Treatment melatonin:
5mg/kg，20mg/kg
(3times/week)

0 1 2 3 4 5 6 7 8 week

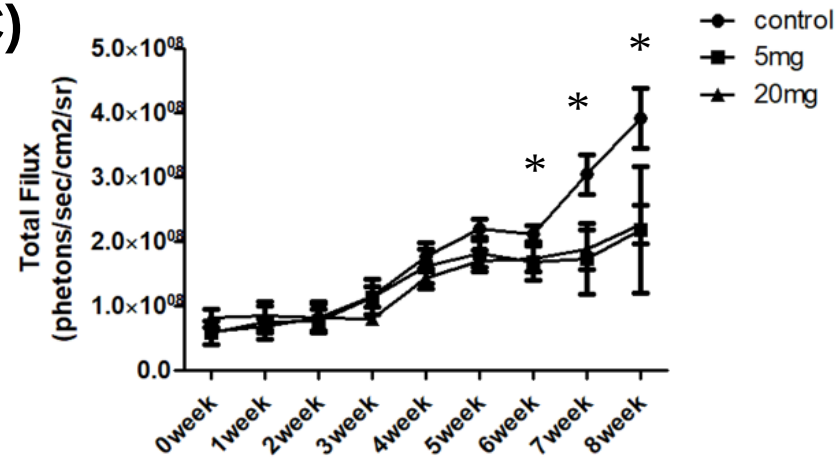
(A)

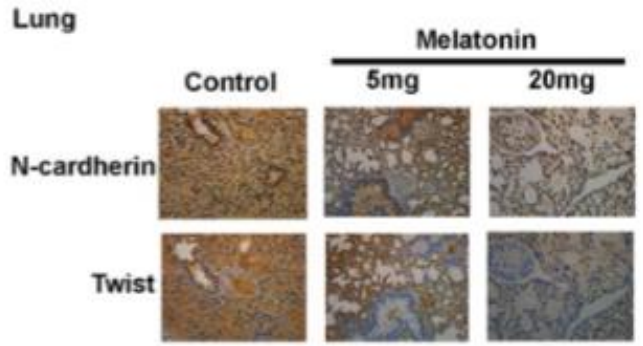


(B)



(C)





➢ **Melatonin inhibits tumor growth and metastasis in the lungs.**

Purposes

Whether melatonin inhibits metastasis and invasion of lung cancer cells?

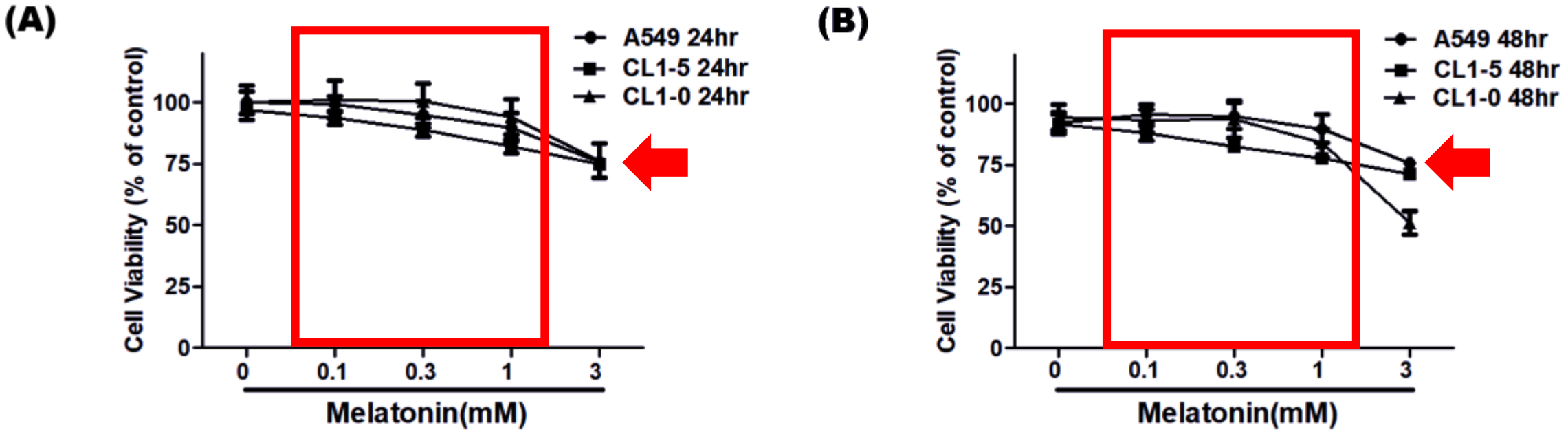
Whether melatonin affect the **EMT** in lung cancer cells and crawling capability?

Which **pathways are involved in melatonin inhibited EMT** of lung cancer cells?

Which **melatonin receptor** are involved in melatonin inhibited **EMT** and **migration** of lung cancer cell ?

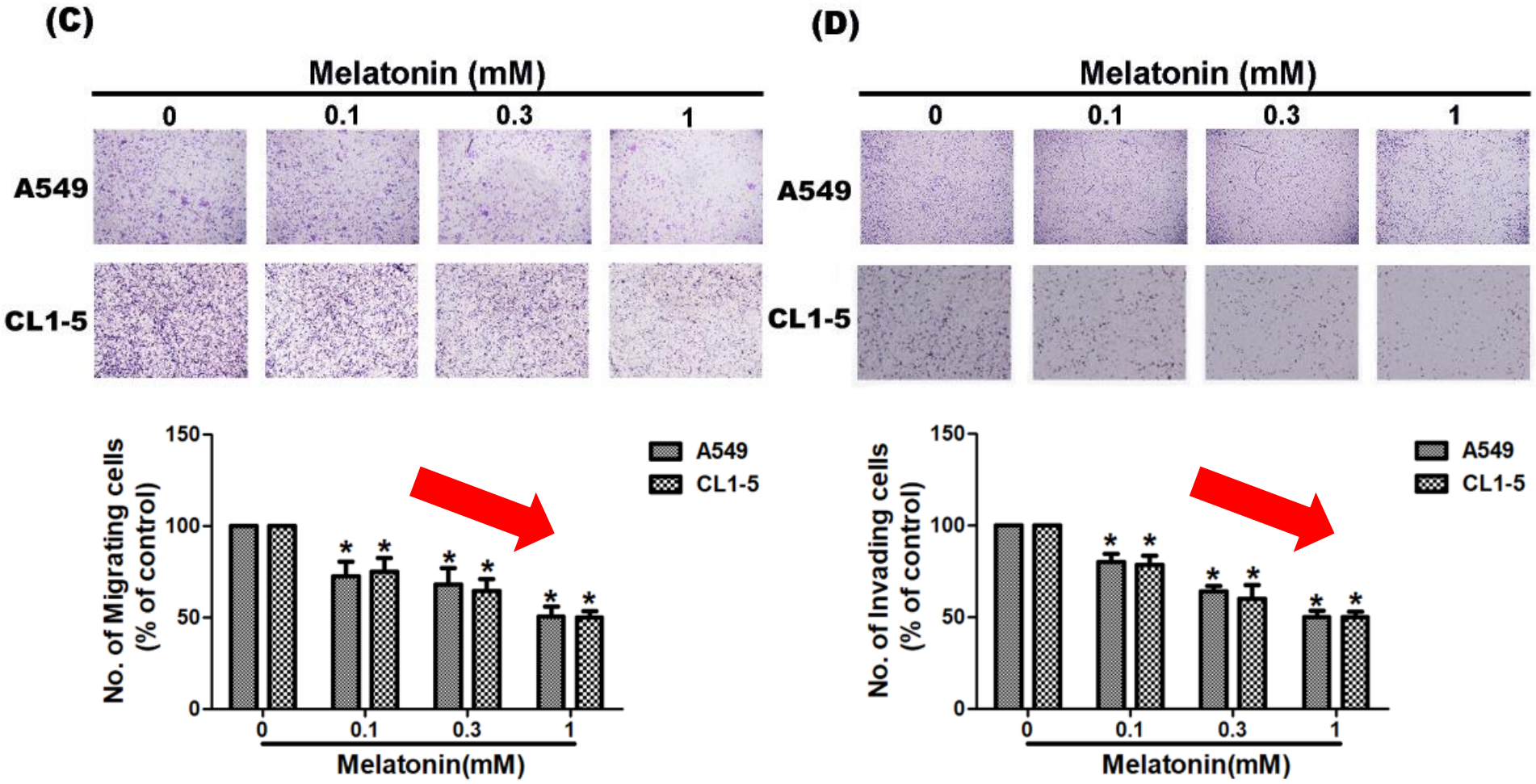
Result

➢ Whether melatonin effects lung cancer cell viability?



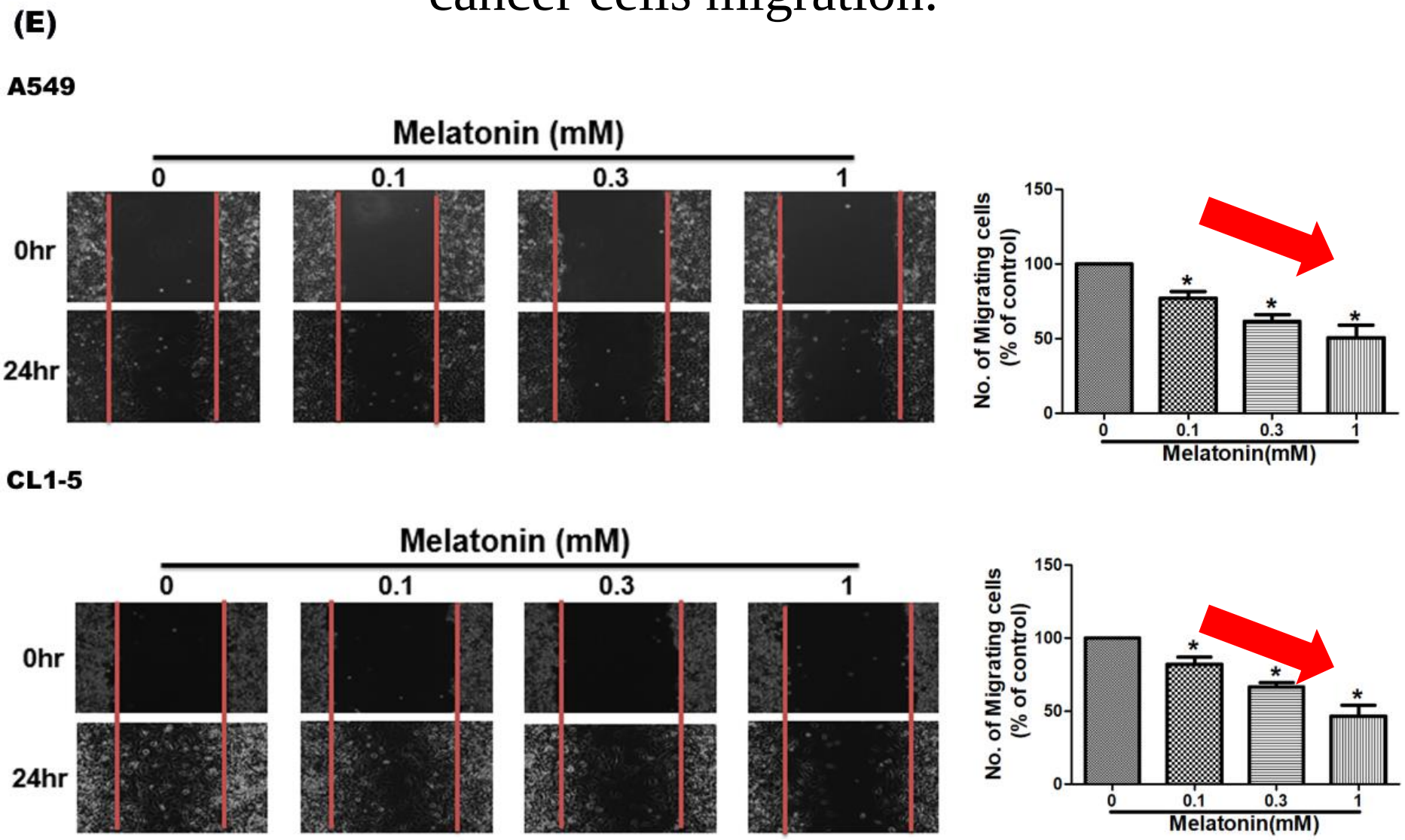
Result

- Whether melatonin dose-dependent effects lung cancer cell migration and invasion?



Result

➢To investigate the ability of melatonin to treat lung cancer cells migration.



➢ **Melatonin inhibits the migration and invasion of lung cancer cells.**

Purposes

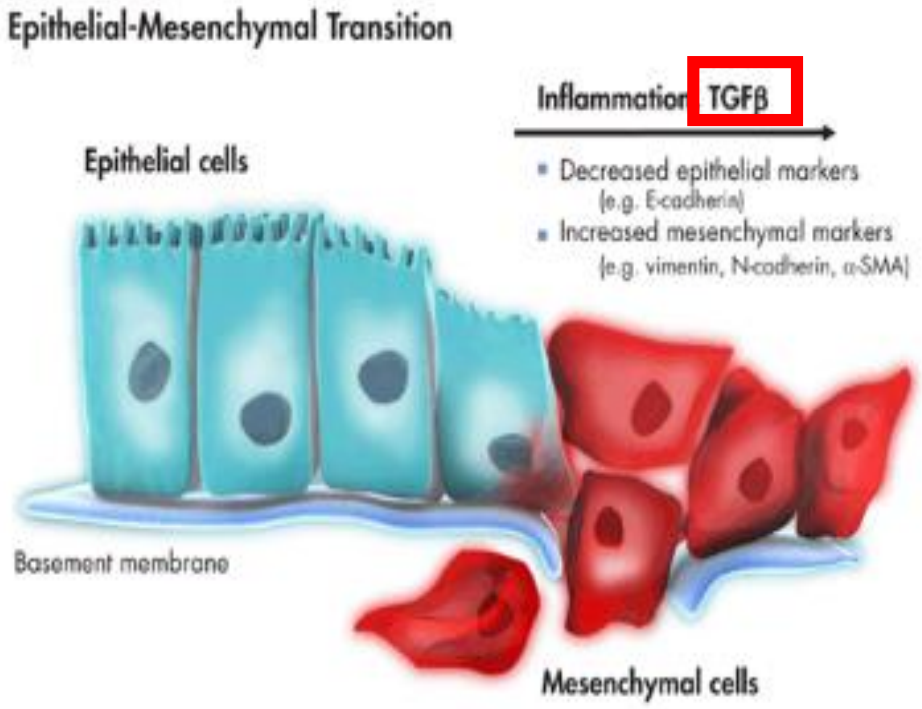
- Whether melatonin inhibits metastasis and invasion of lung cancer cells?
- **Whether melatonin affect the EMT in lung cancer cells and crawling capability?**
- Which pathways are involved in melatonin inhibited EMT of lung cancer cells?
- Which melatonin receptor are involved in melatonin inhibited EMT and migration of lung cancer cell ?

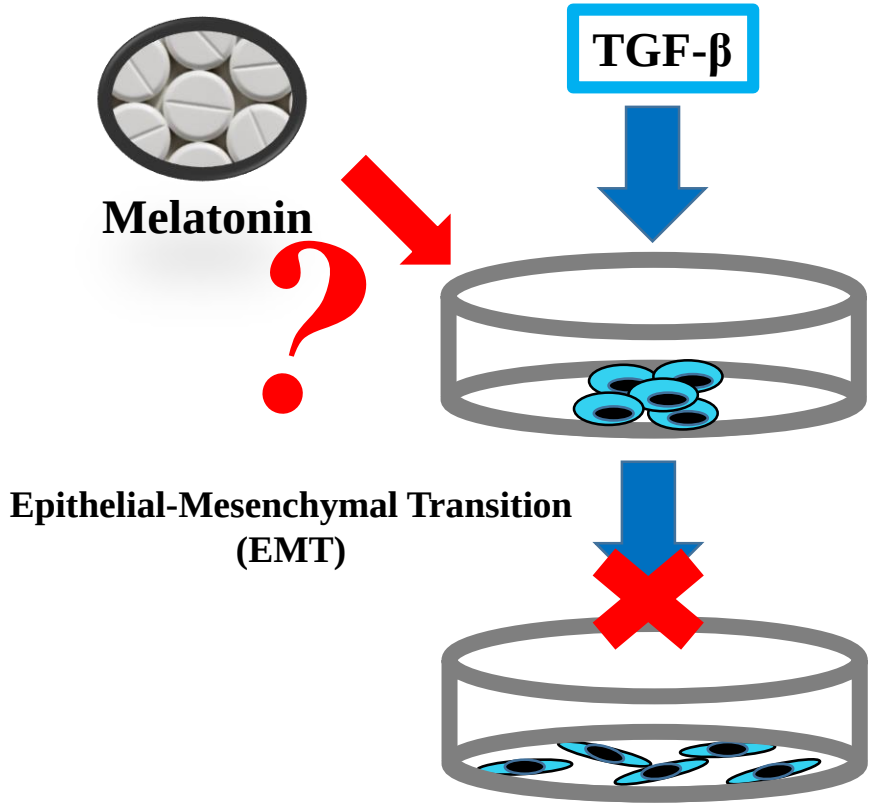
Cell Migration
Volume 294 of the series Methods in Molecular Biology™ pp 69-77

Cell-Scatter Assay

Hong-Chen Chen

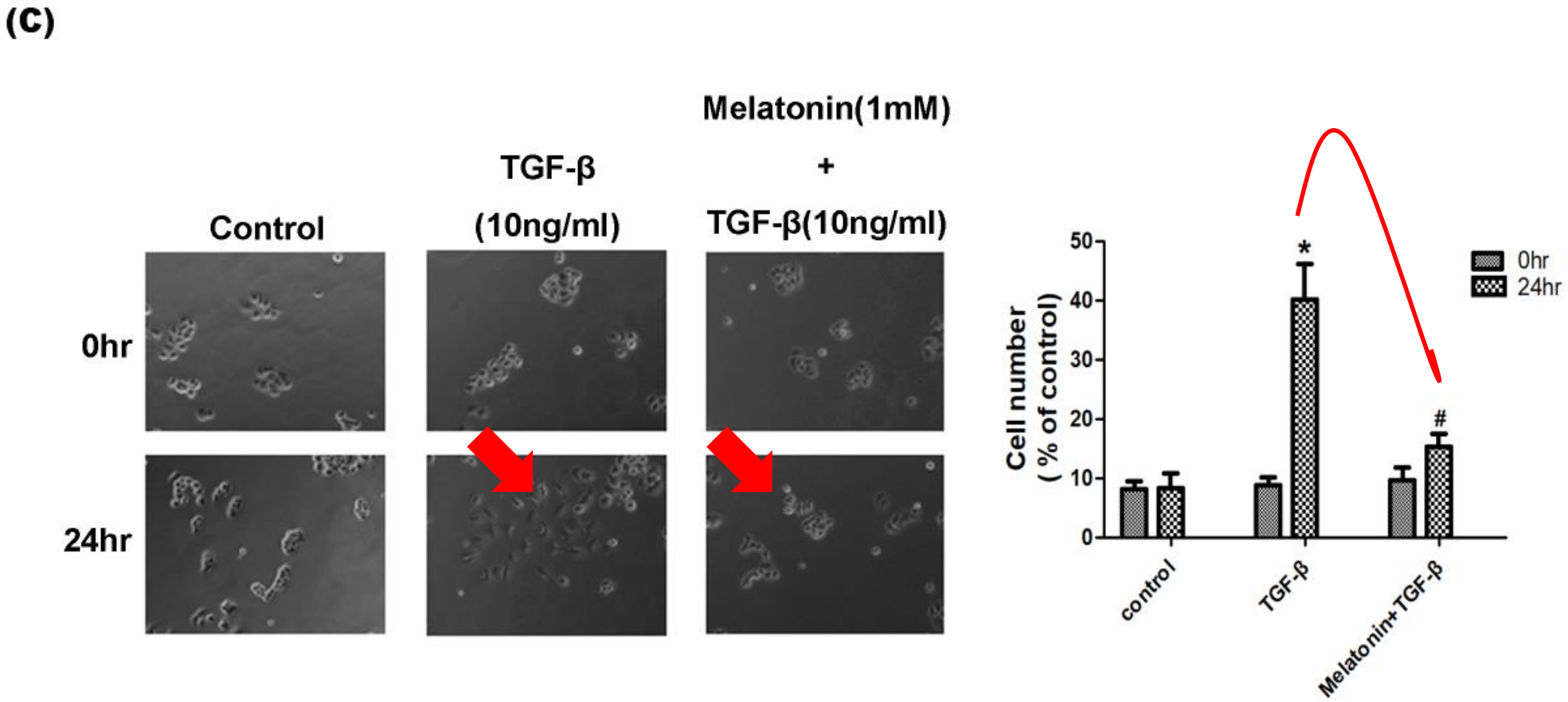
The scatter assay has been used for studying epithelial-mesenchymal transition and for detecting factors able to induce migratory behavior of cells.



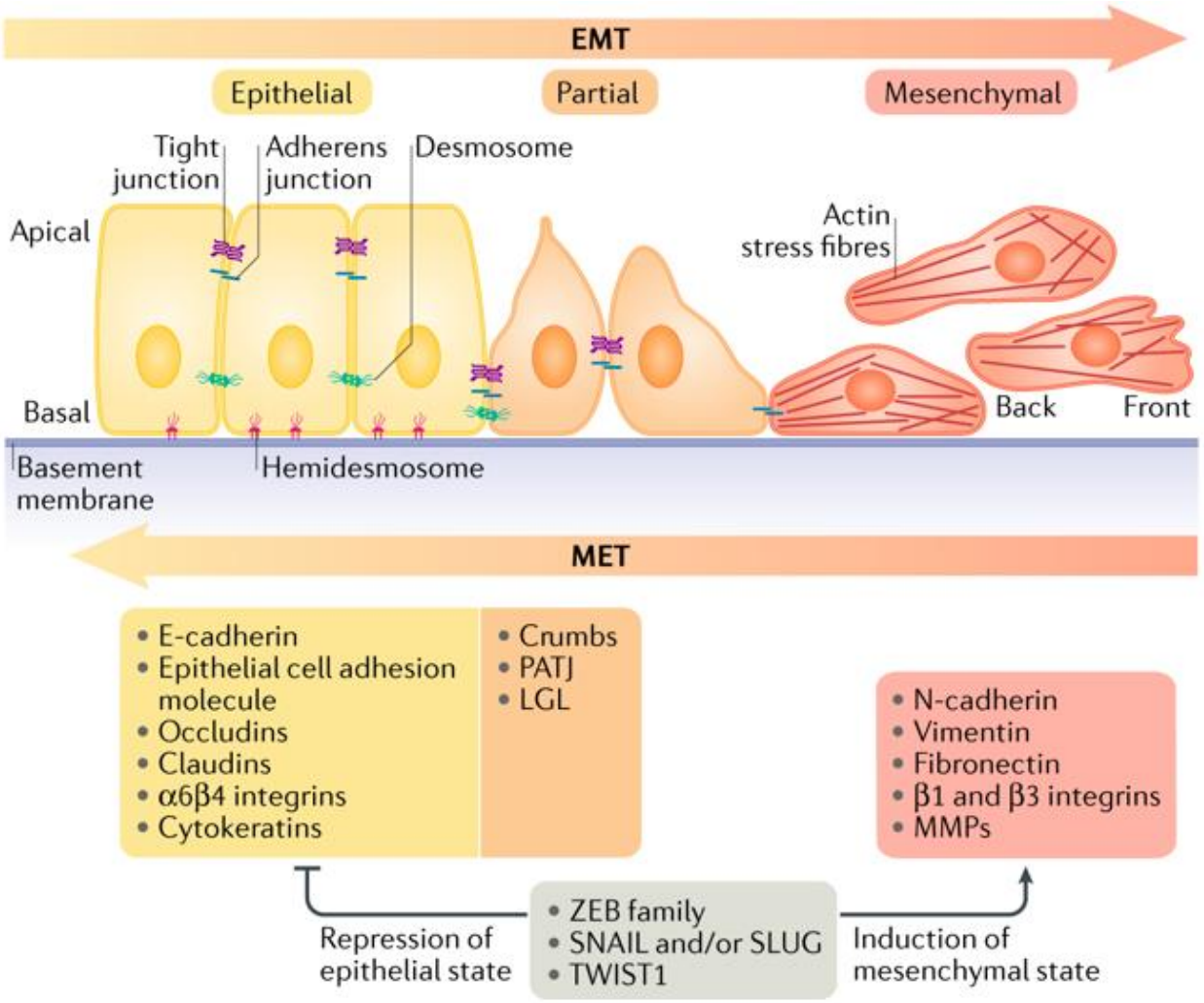


Result

- Whether melatonin affects cell TGF-β induced EMT alteration of cell morphology and migration in CL1-0 cells?



Epithelial Mesenchymal Transition, EMT



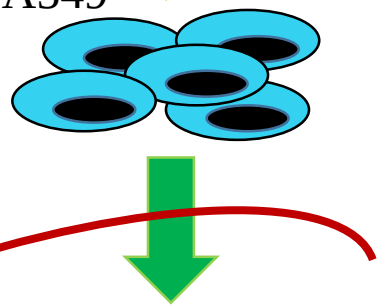
Anushka Dongre, 2018

Result

- Whether melatonin suppresses cell mobility by modulating EMT maker in lung cancer cell?

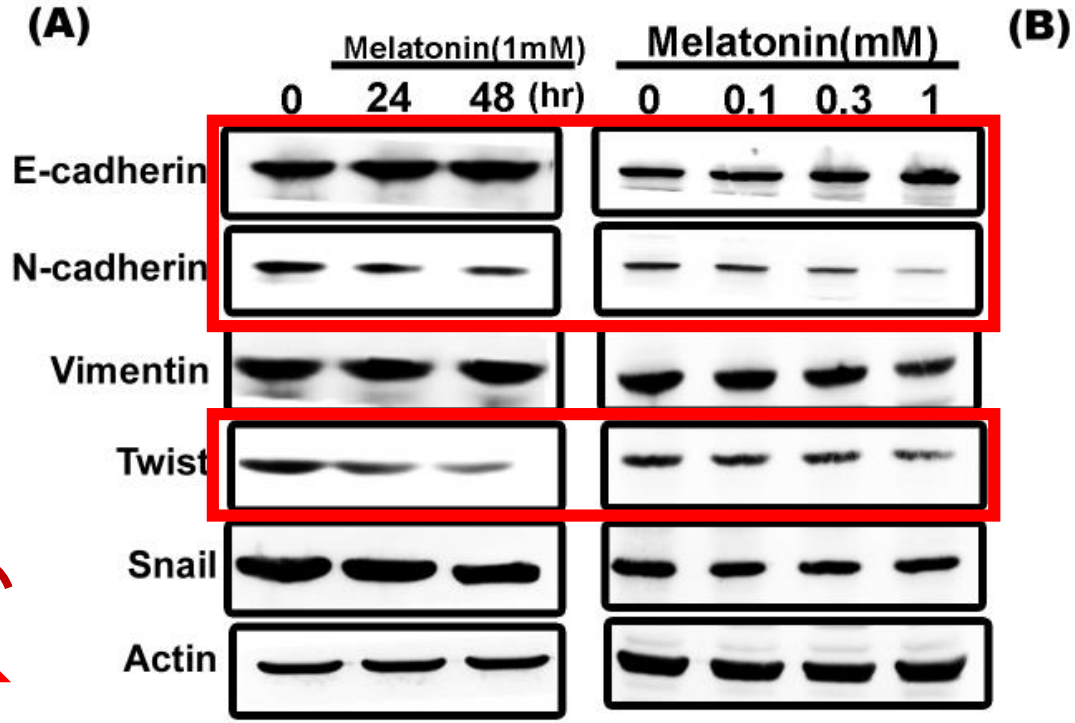
Melatonin

A549

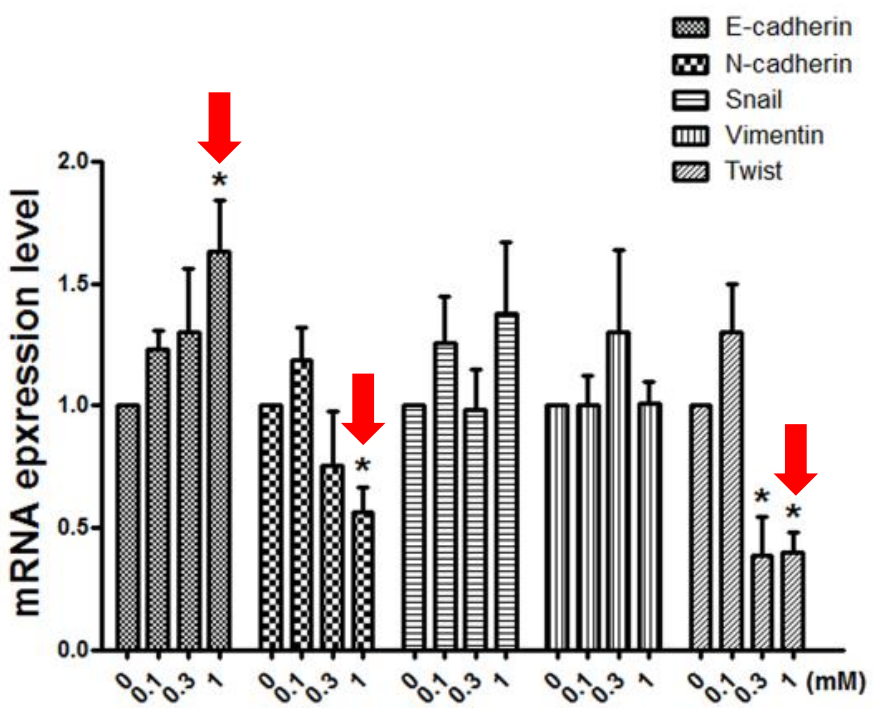


E-cadherin ↑
N-cadherin ↓
Twist ↓

Western blot



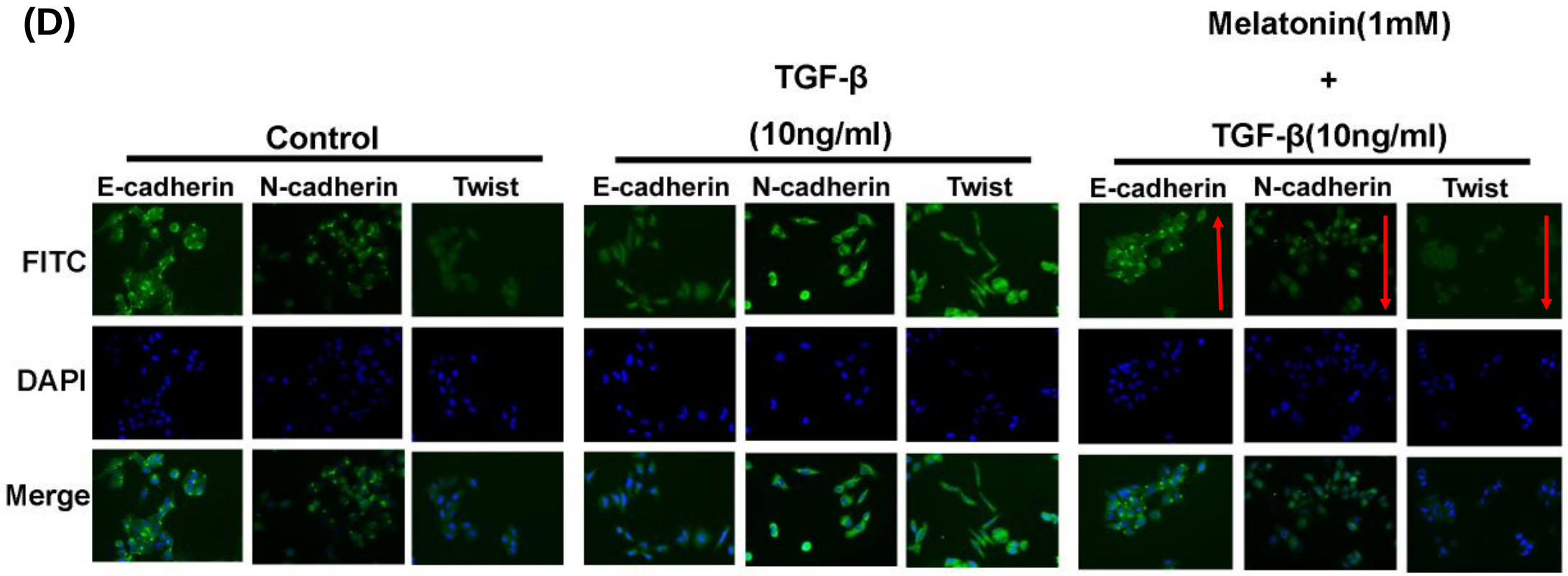
q-PCR



- **That melatonin affects EMT markers expression in lung cancer cells, such as E-cadherin, N-cadherin and Twist.**

Result

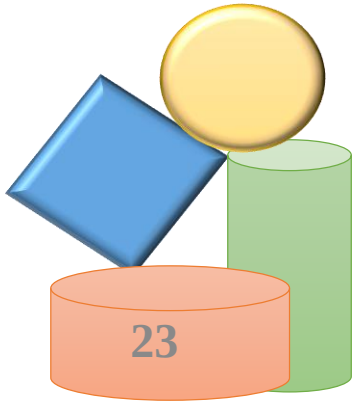
➢To investigate the EMT markers change after melatonin treatment.



➢ **That melatonin affects E-cadherin, N-cadherin and Twist expression and cell morphology and migration in lung cancer cells.**

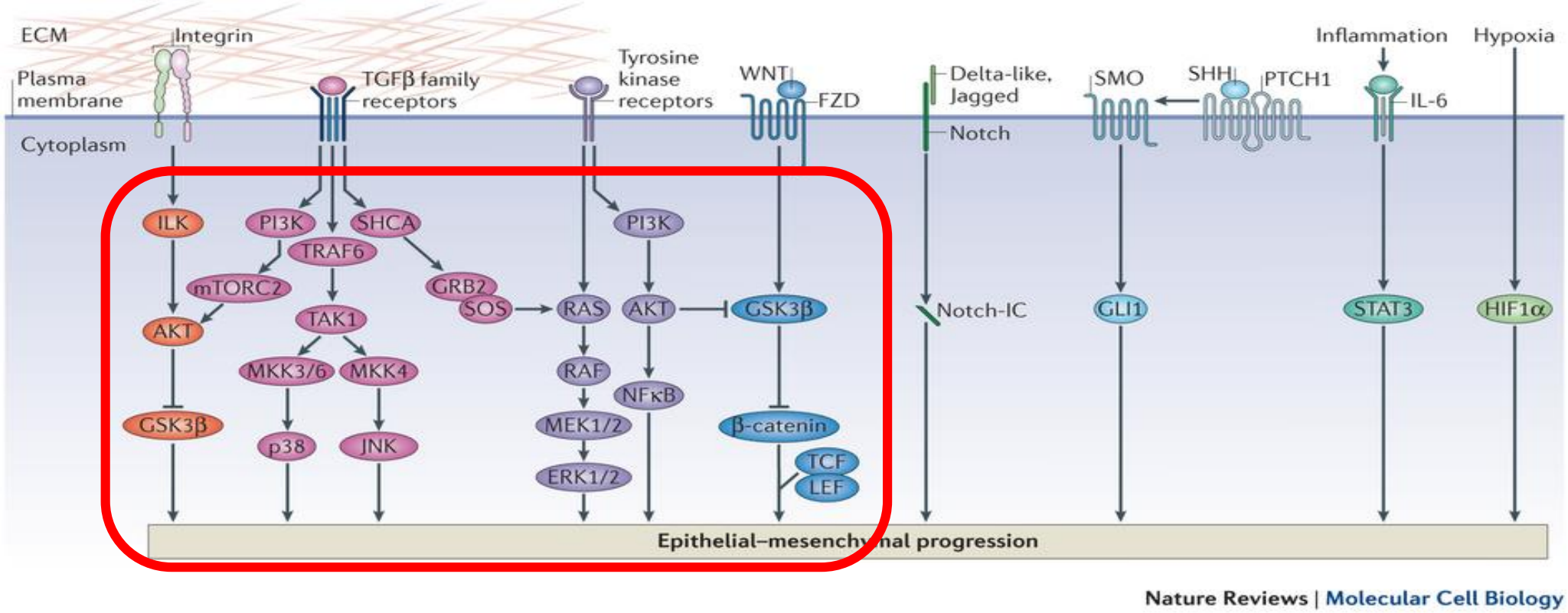
Result

- Whether melatonin inhibits metastasis and invasion of lung cancer cells?
- Whether melatonin affect the EMT in lung cancer cells and crawling capability?
- **Which pathways are involved in melatonin inhibited EMT of lung cancer cells?**
- Which melatonin receptor are involved in melatonin inhibited EMT and migration of lung cancer cell ?



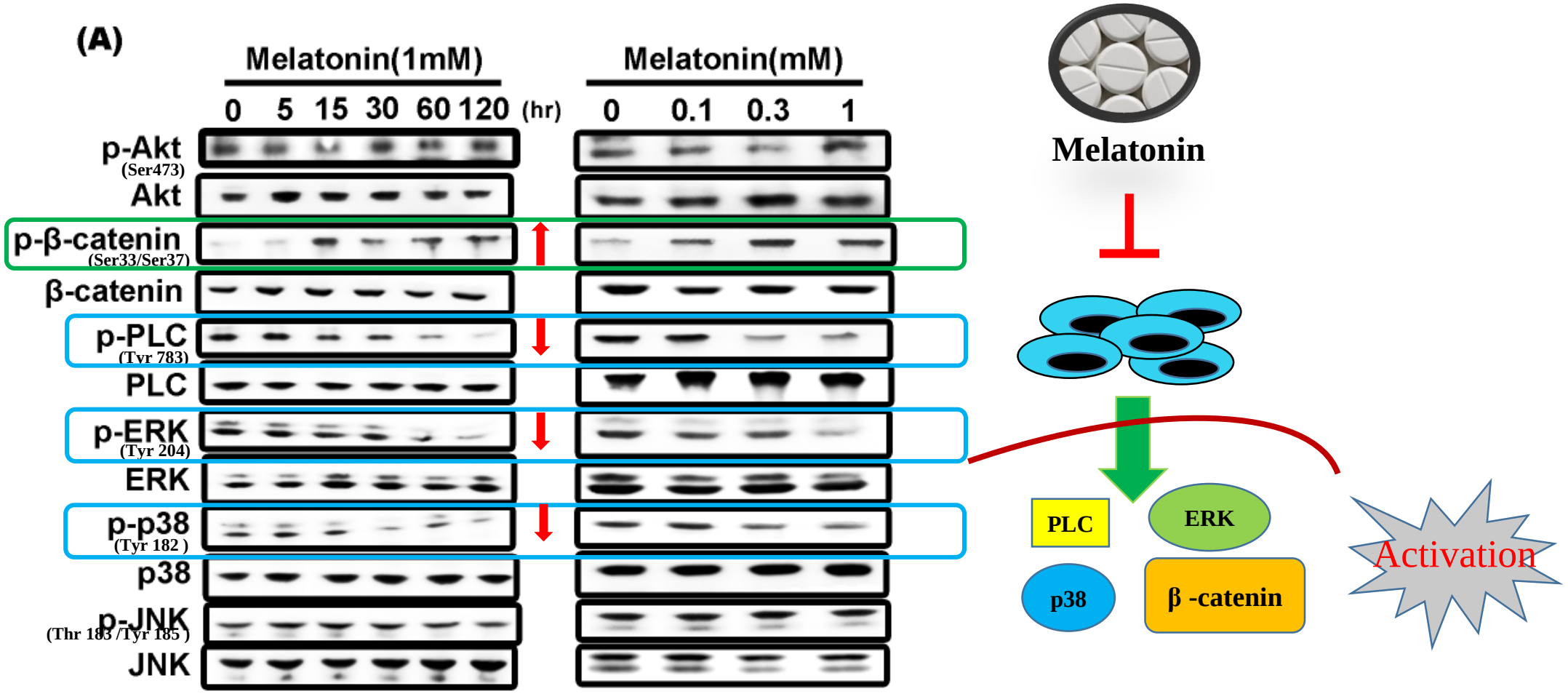
Result

➢Which signaling pathway are involved in melatonin regulated EMT?



Result

➢ Which pathways are involved in melatonin inhibited EMT of lung cancer cell ?



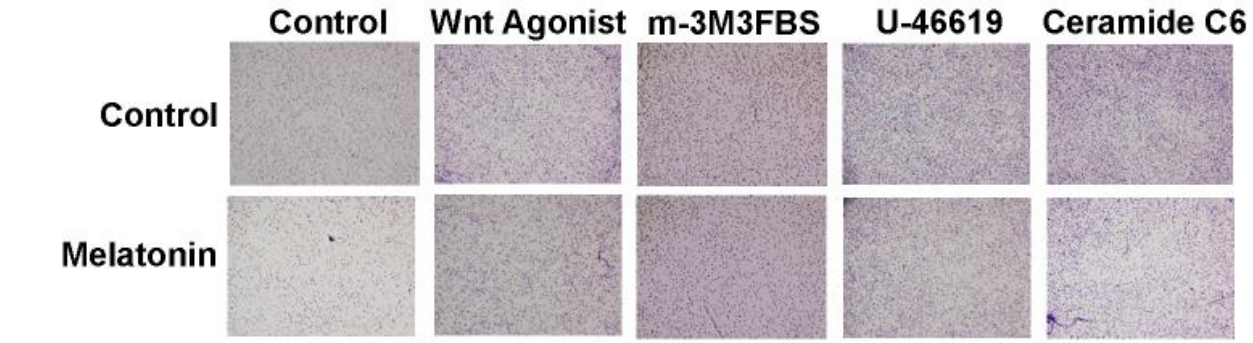
Result

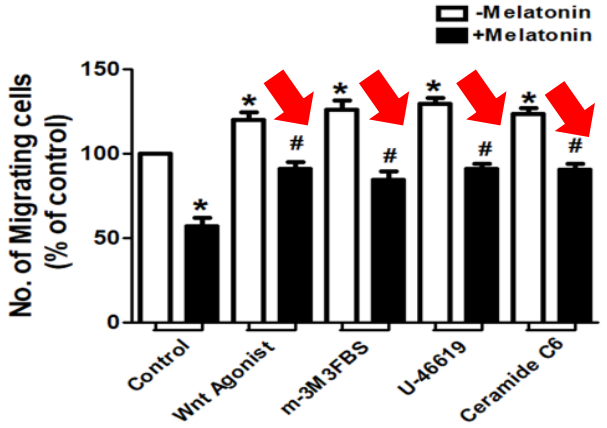
➢Whether these pathways activators were involved in cell mobility regulation ?

Wnt Agonist : β-catenin (+)
m-3M3FBS : PLC (+)
U-46619 : p38 (+)
Ceramide C6 : ERK (+)

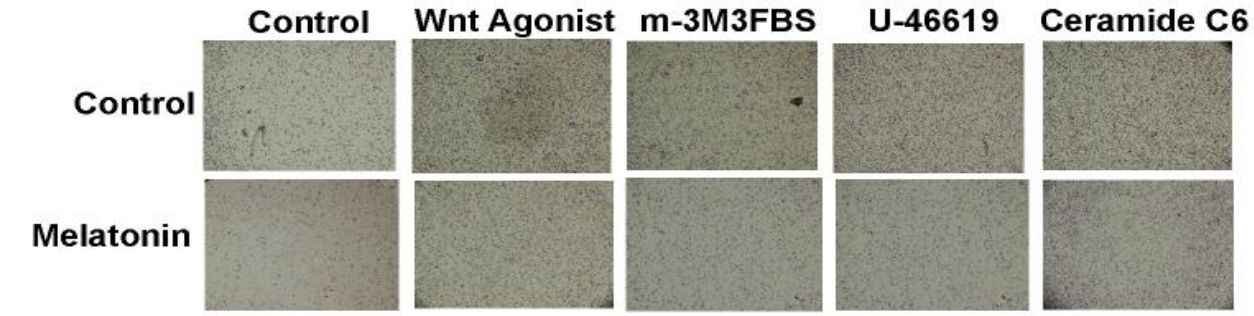
(C)

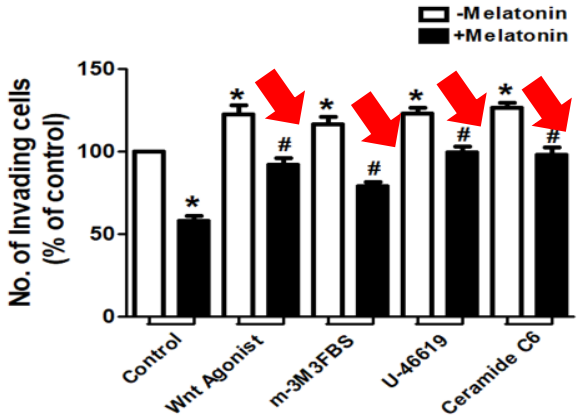
Migration





Invasion

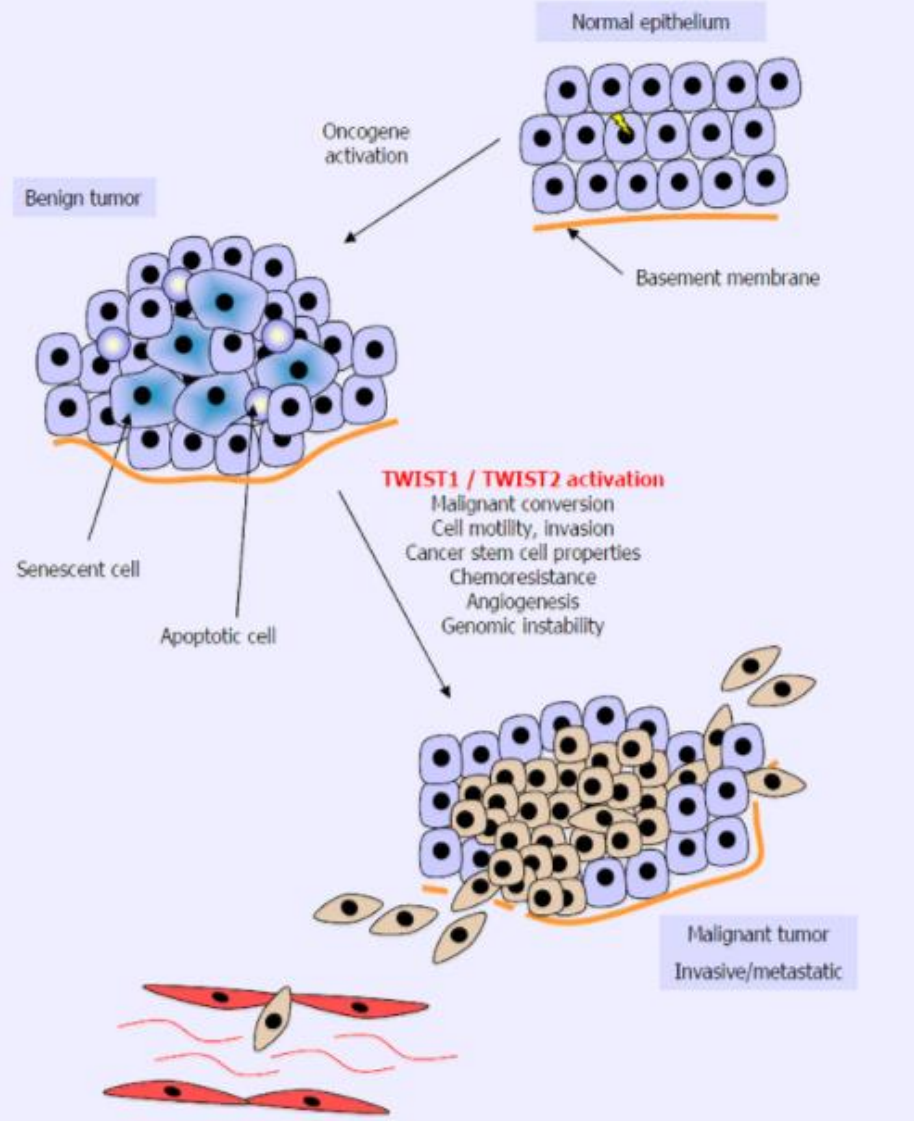




The PLC, p38, ERK, β –catenin pathways are involved in the melatonin-mediated suppression cell motility.

Result

➢ Whether melatonin suppressed Twist by regulating PLC / p38 / ERK / β-catenin pathways?



EMT: Giving senescence a TWIST

Cell Migration Gateway (August 2008) | doi:10.1038/cmg076

TWIST transcription factors cooperate induce epithelial-mesenchymal transition, thus favoring tumor progression and dissemination.

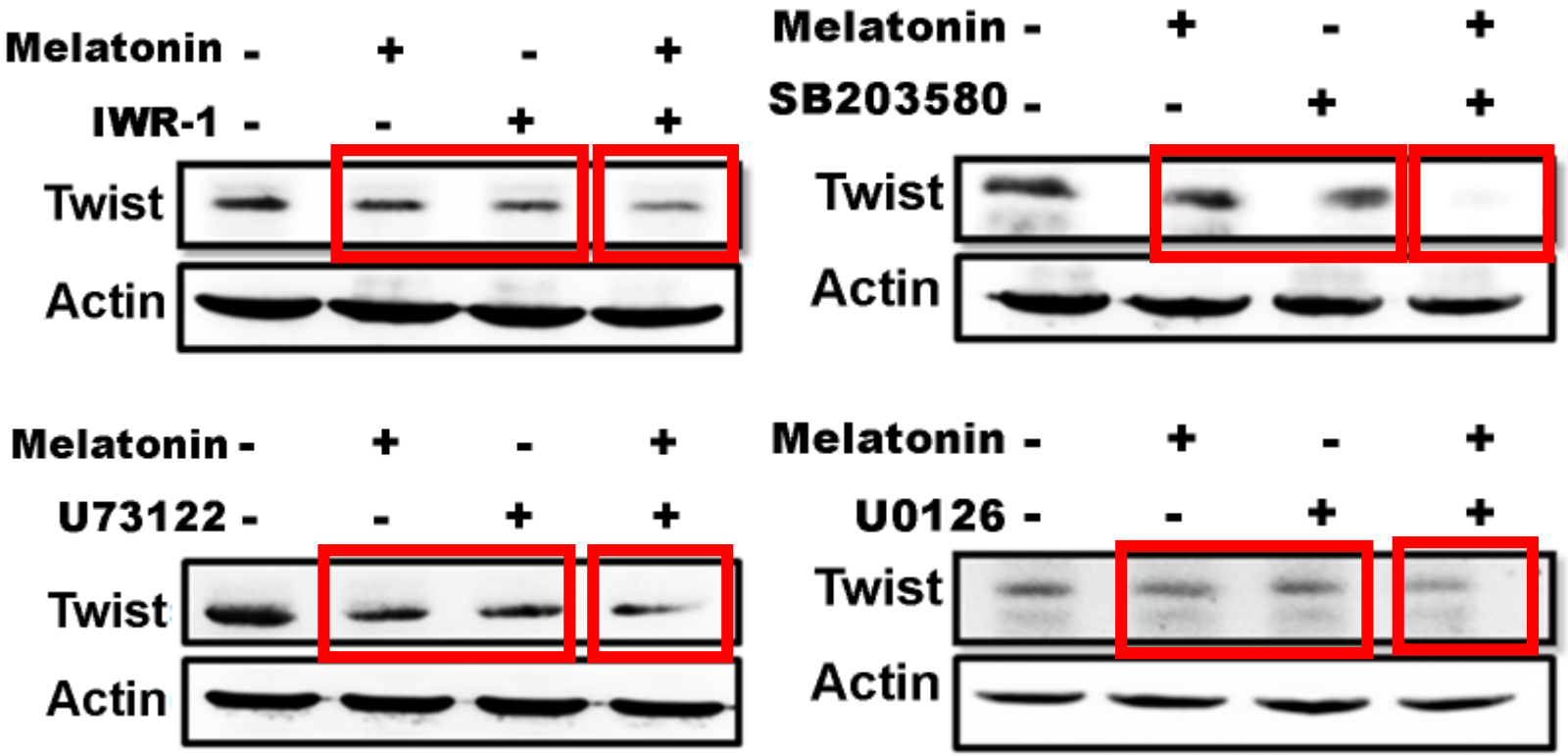
Result

- The treated with inhibitors of PLC / P38 / ERK / β-catenin and combined with melatonin will inhibited twist protein and mRNA expression.

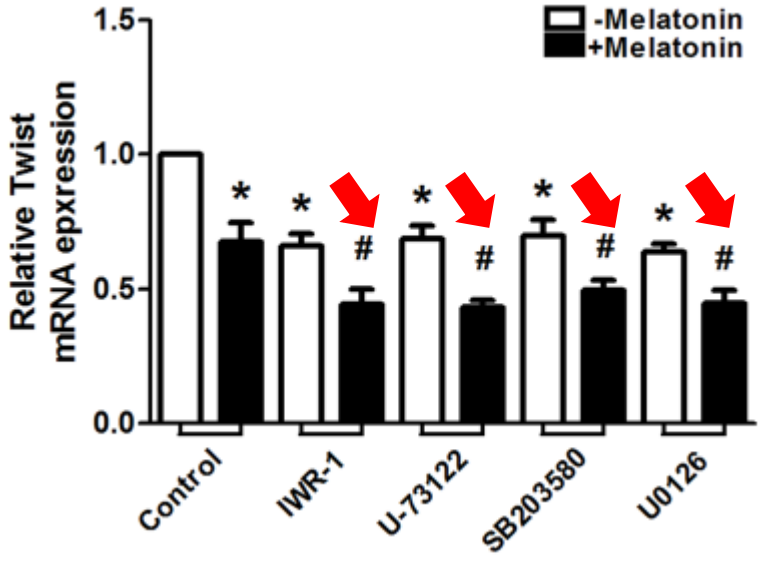
IWR-1 : β-catenin (-)
U73122 : PLC (-)
SB203580 : p38 (-)
U0126 : ERK (-)

(E)

Western blot



qPCR



➢ **The PLC, p38, ERK, β-catenin pathway were involved in melatonin regulated Twist.**

Result

Whether melatonin inhibits metastasis and invasion of lung cancer cells?

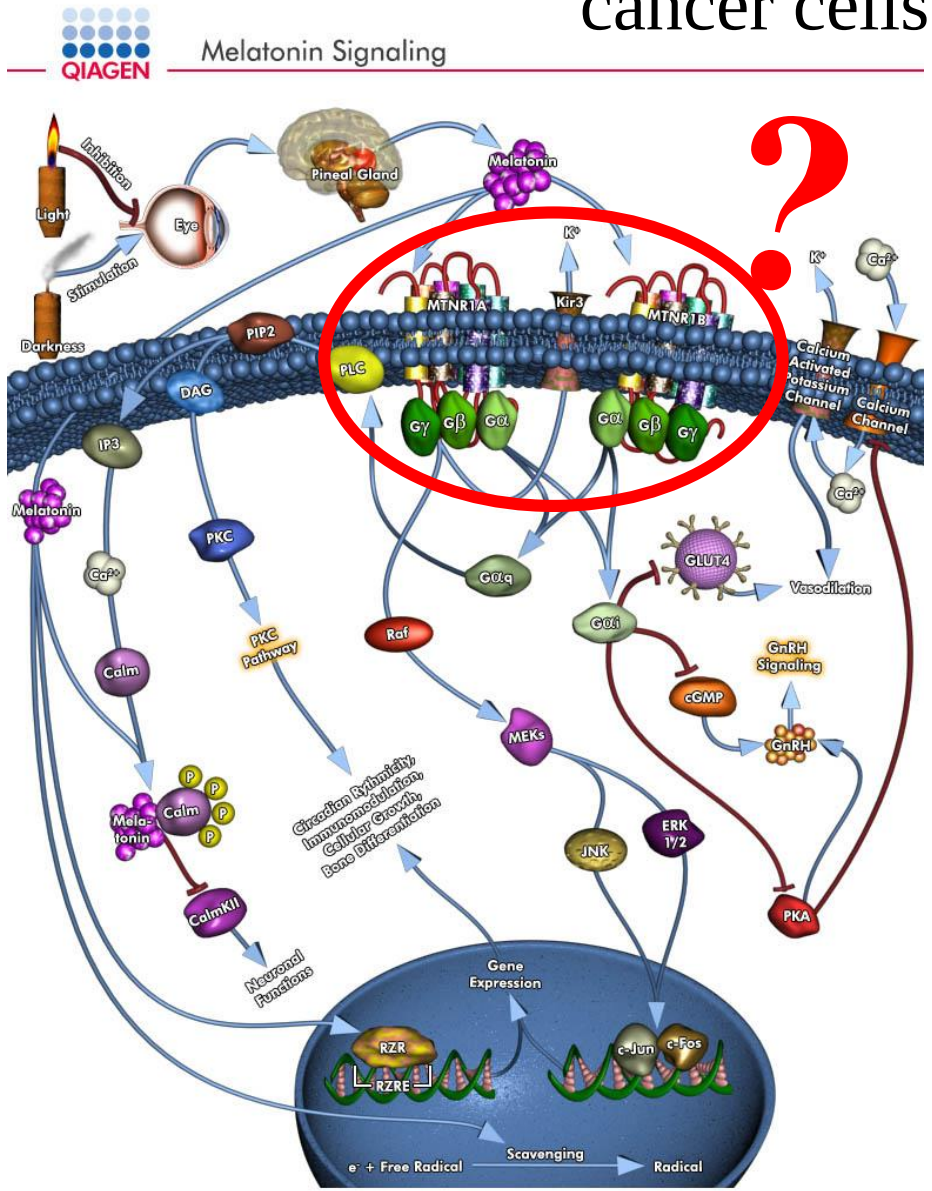
Whether melatonin affect the EMT in lung cancer cells and crawling capability?

Which pathways are involved in melatonin inhibited EMT of lung cancer cells?

Which melatonin receptor are involved in melatonin inhibited EMT and migration of lung cancer cell ?

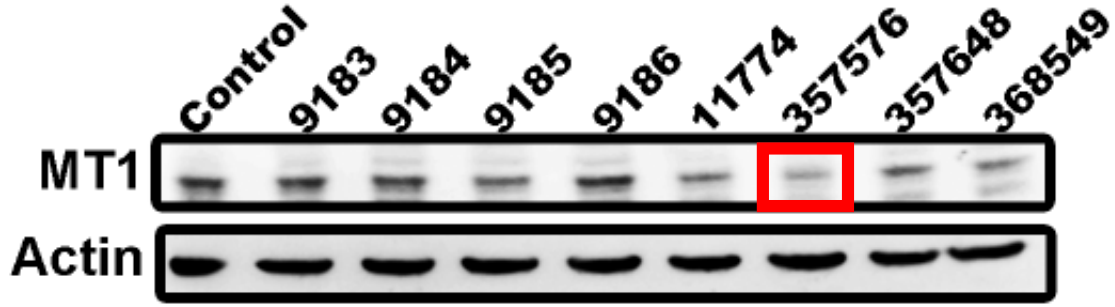
Result

➢Which melatonin receptor regulate the function of lung cancer cells?

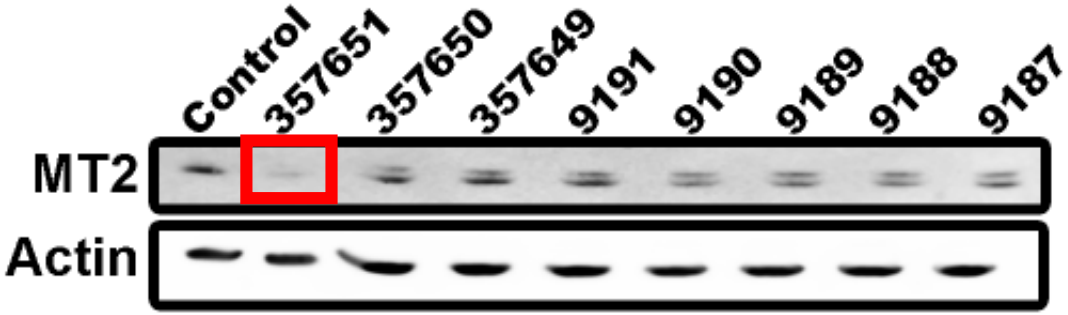


(A)

shRNA

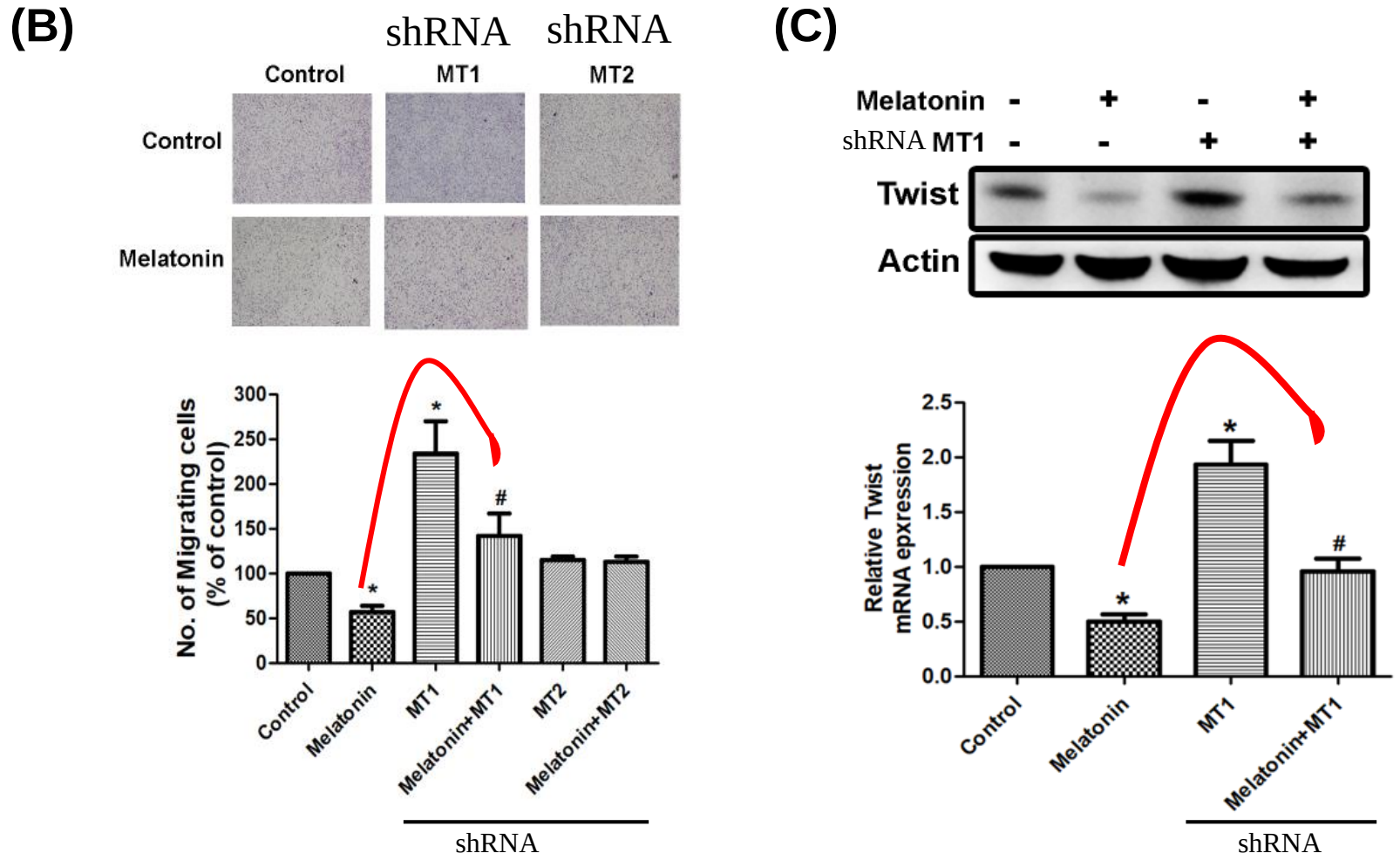


shRNA



Result

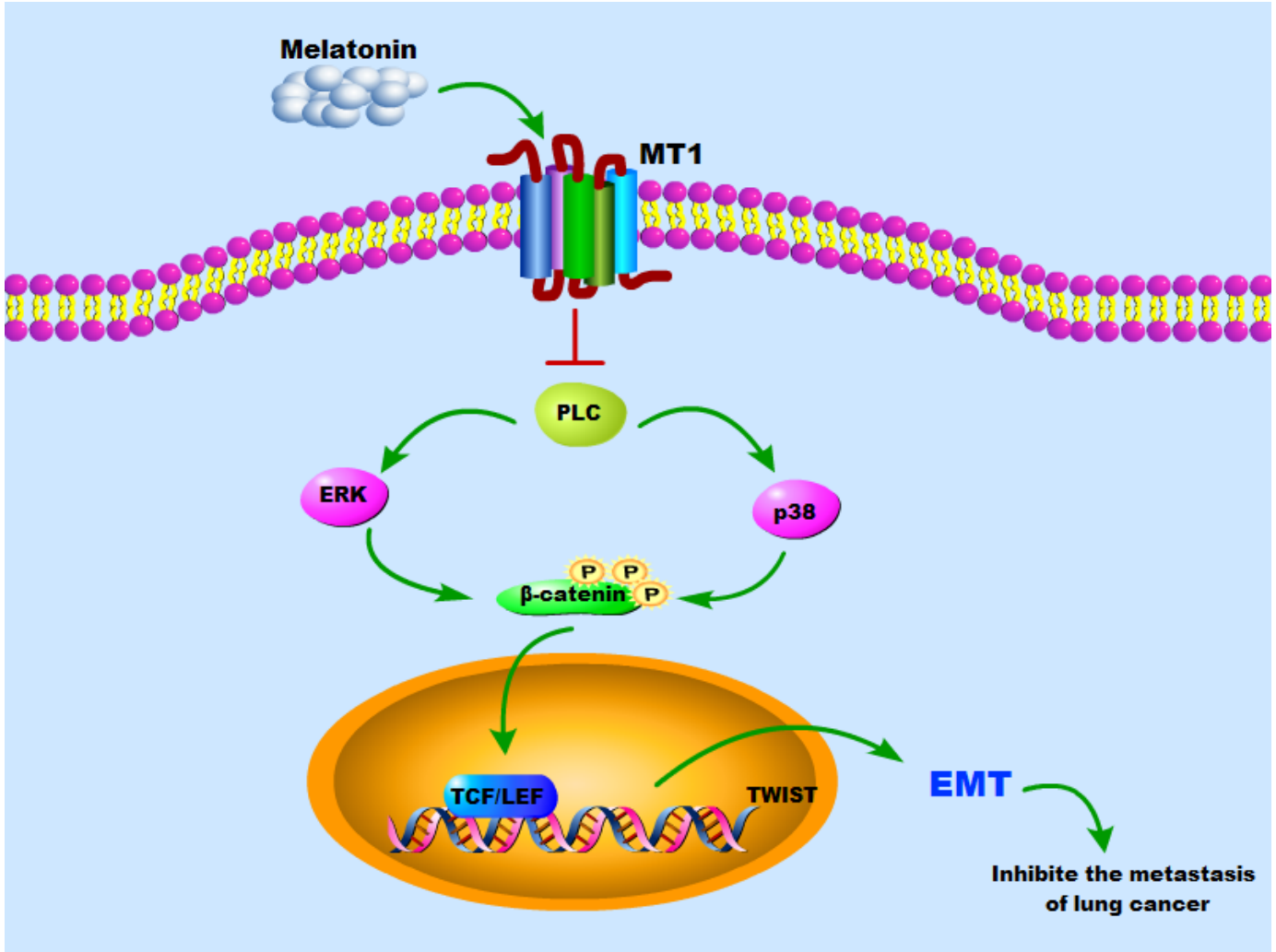
➢Whether Melatonin affects the migration ability of lung cancer cells through MT1 (357576) and MT2 (357651)?



Melatonin through MT1 receptor inhibits lung cancer cells migration and Twist.

Conclusion

- Melatonin reduces the Twist and migratory activity of lung cancer cells by suppresses Twist expression via MT1 regulate PLC/p38/ERK/β-catenin pathway.



Acknowledgment

- Fu-Jen Catholic University
- China Medical University
  Chih-Hsin Tang, Pei-Chen Chiou
- Shin-Kong Wu Ho-Su Memorial Hospital
  Po-Chun Chen

  Joan Chen, Liang-Wei Lin
- Chung Shan Medical University,
  Shun-Fa Yang