

# 난자특이발현 유전자인 Diva의 난소암세포주에서의 기능에 대한 연구

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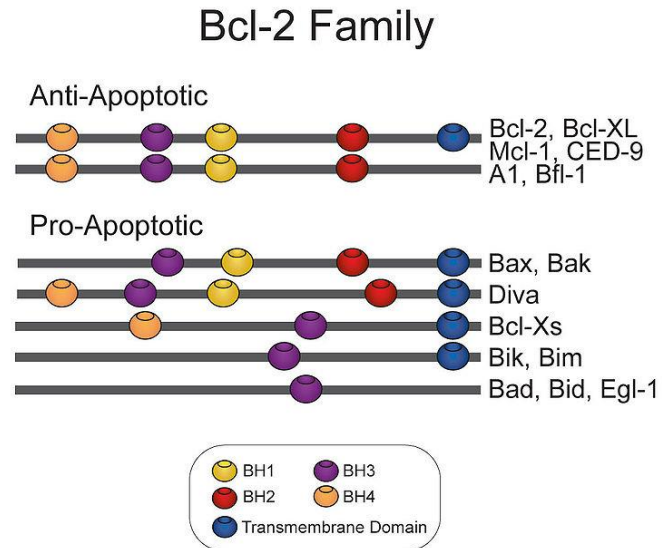
Lab. of Molecular Reproductive Physiology

Professor. Kyung-Ah Lee

# Background - What is Diva ?

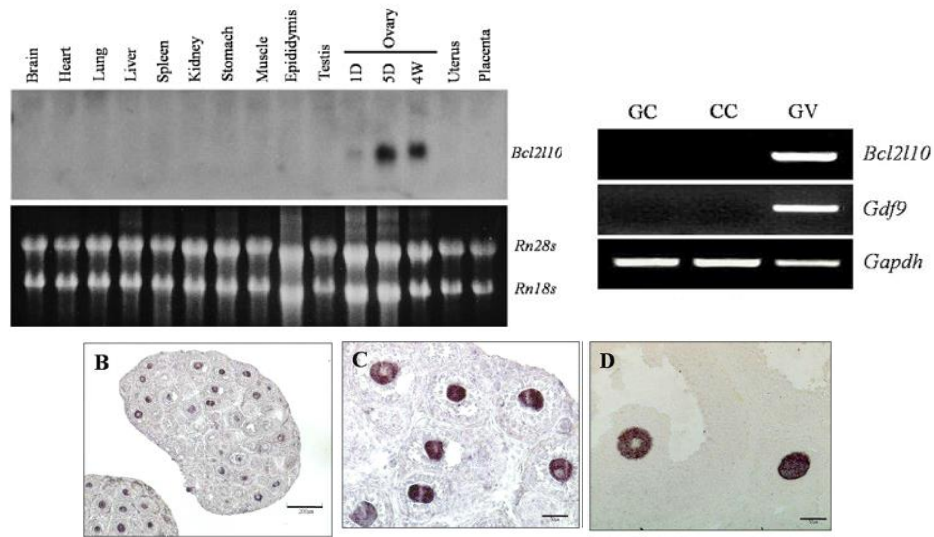
## Diva (Bcl2l10, Bcl-b, Boo)

- Bcl2 family proteins are characterized by the presence of one or more conserved domains called BH (for 'BCL2 homology') domains (BH1–4).
- B cell lymphoma-2 (BCL2) family proteins are key regulators of the apoptotic process.
- Diva is encoded by this gene belongs to the BCL-2 protein family.
- Diva has contradictory functions (as anti- or pro-apoptotic regulators) in apoptosis.

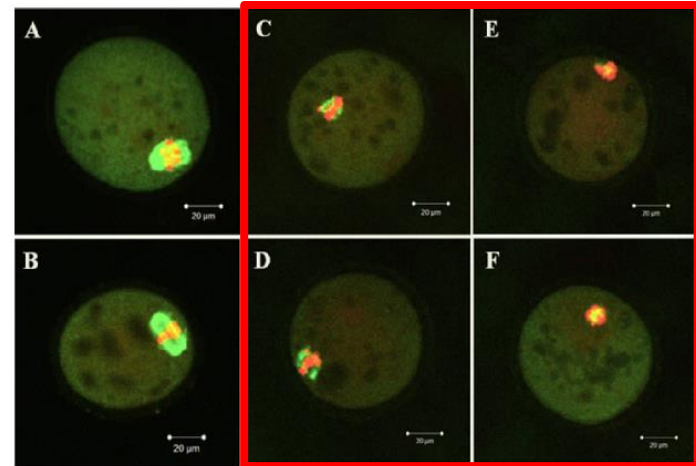
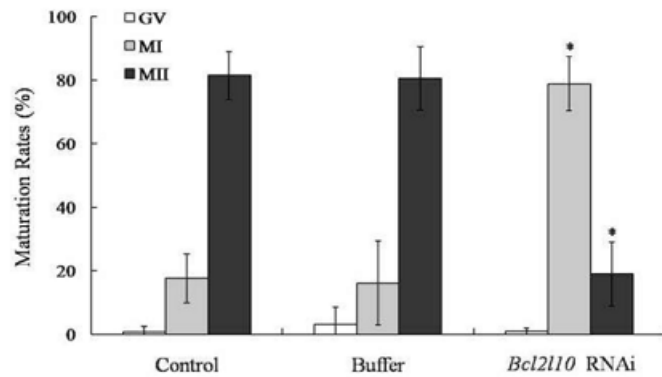


(Chao & Korsmeyer 1998)

# Background - Diva is essential for oocyte maturation



## Divia RNAi



(Yoon et al., 2009)

# Background - Diva is a key regulator of Aurora A in oocytes

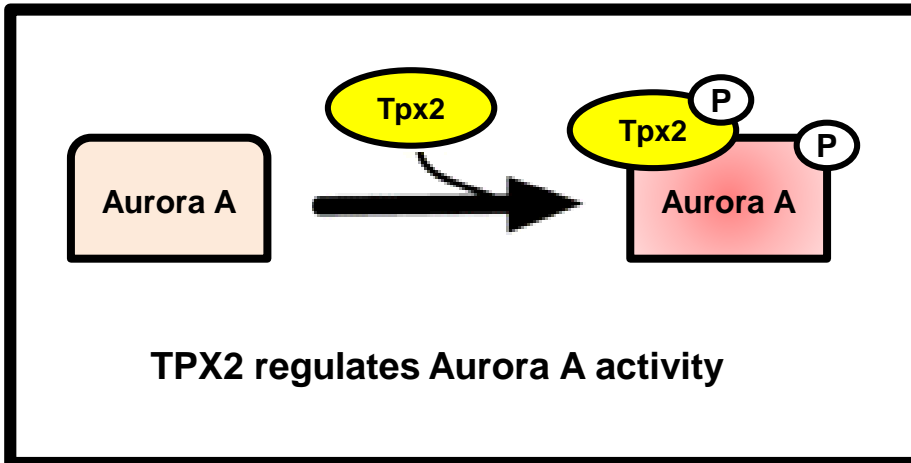
We conducted the study to investigate the downstream genes of Diva in the oocytes by using microarray analysis.

## genes down-regulated more than 2 folds by Diva RNAi

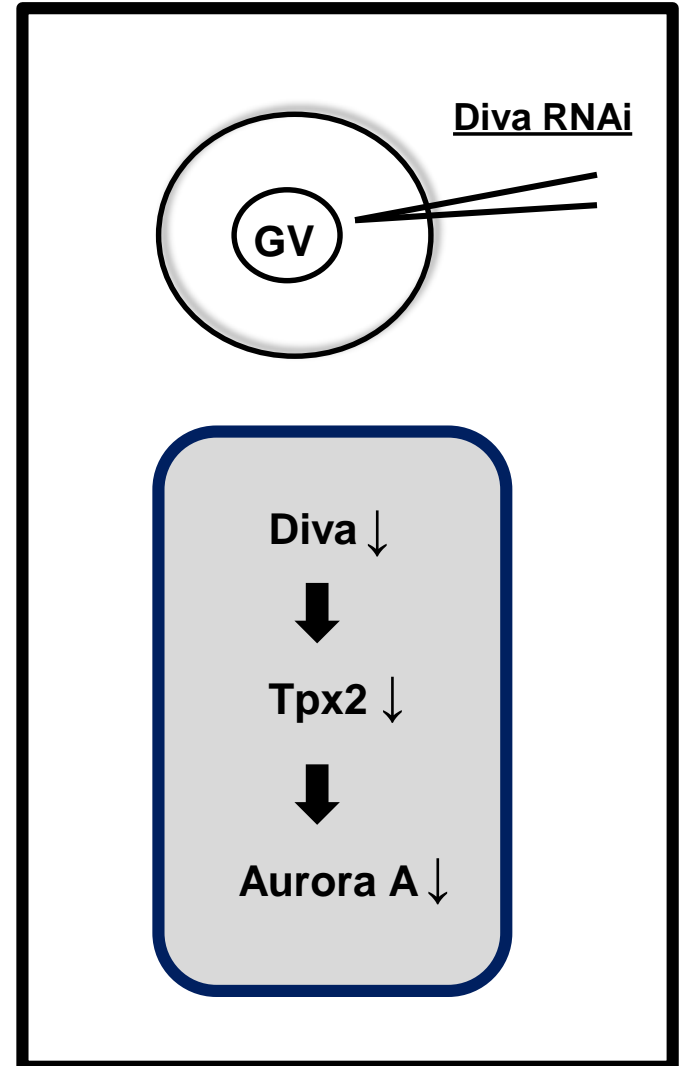
Genes	Gene titles	Fold changes
<b>Tpx2</b>	TPX2, microtubule-associated protein homolog(Xenopus laevis)	-16.1
Rbm12b	RNA binding motif protein 12B	-15.3
Ptp4a1	Protein tyrosine phosphatase 4a1-like	-14.6
Ranbp2	RAN binding protein 2	-10.1
Eeal	Early endosome antigen 1	-9.3
Arid4a	AT rich interactive domain 4A (RBP1-like)	-9.1
Cep192	Centrosomal protein 192	-8.2
Kif20b	Kinesin family member 20B	-7.9
Psip1	PC4 and SFRS1 interacting protein 1	-7.8
Atad2b	ATPase family, AAA domain containing 2B	-7.5

(Kim et al., 2011)

# Background - Diva is a key regulator of Aurora A in oocytes

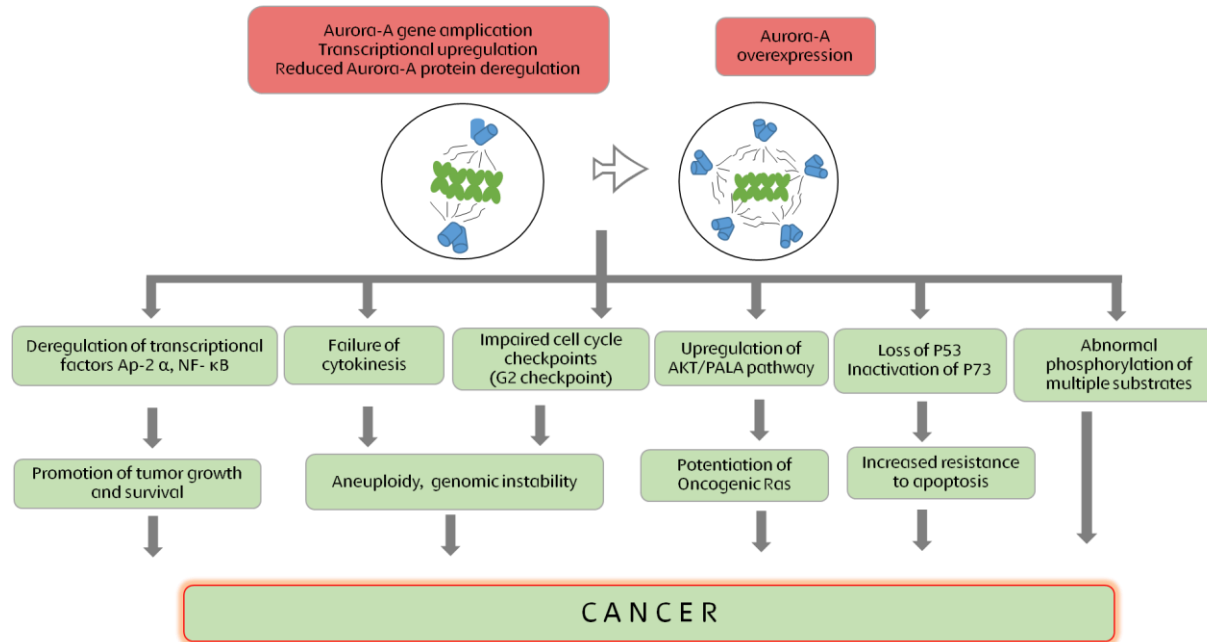


(Richard et al., 2003; Alexis & Fanni 2007)



# Background - Aurora kinase A is cancer therapy target as oncogene

## Aurora kinase A



- Overexpression of Aurora A is common in many types of solid tumor.
- Aurora A is associated with chromosomal instability, aneuploid, supernumerary centrosomes, multipolar spindle.
- These properties have led Aurora A to be considered a high value target for development of cancer therapeutics.

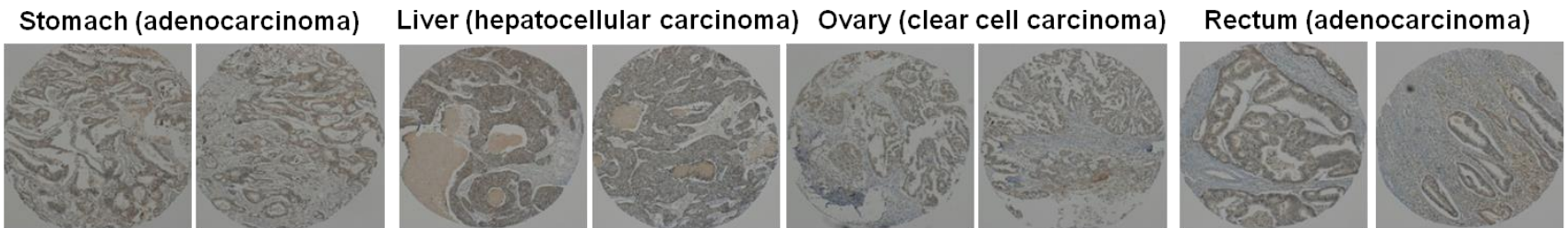
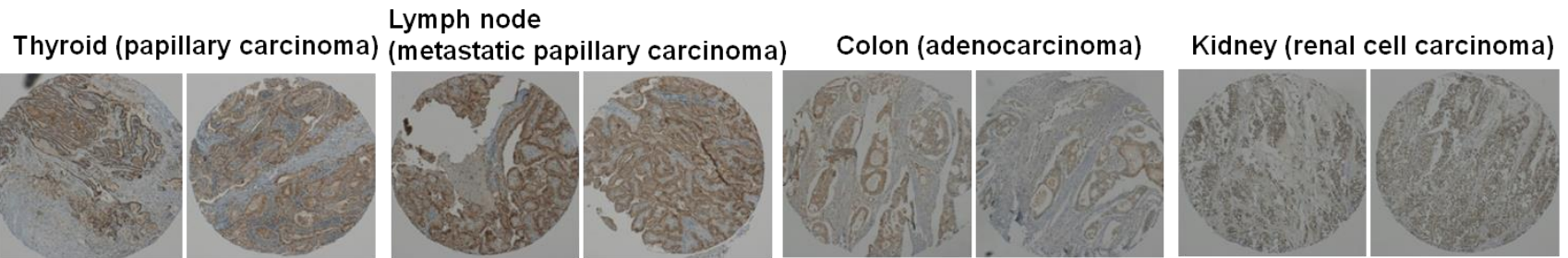
(Nikonova et al., 2013)

# Objectives



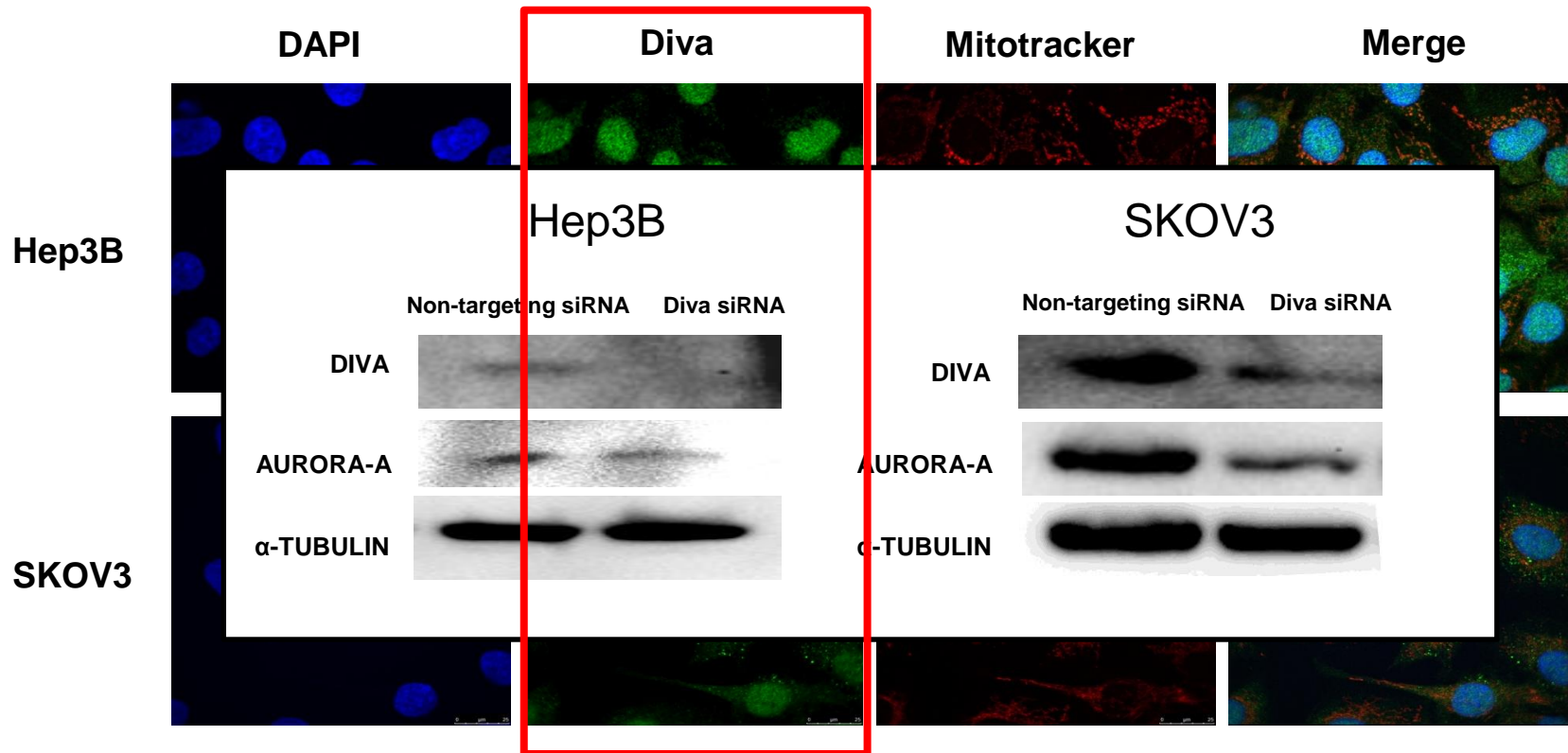
- To determine the regulatory role of Diva on Aurora A expression in cancer cells.
- To investigate the effects of Dive RNAi on the characteristics of cancer cells.

# Result 1. Tissue Microarray data showed that Diva protein expressed in various human cancer tissues

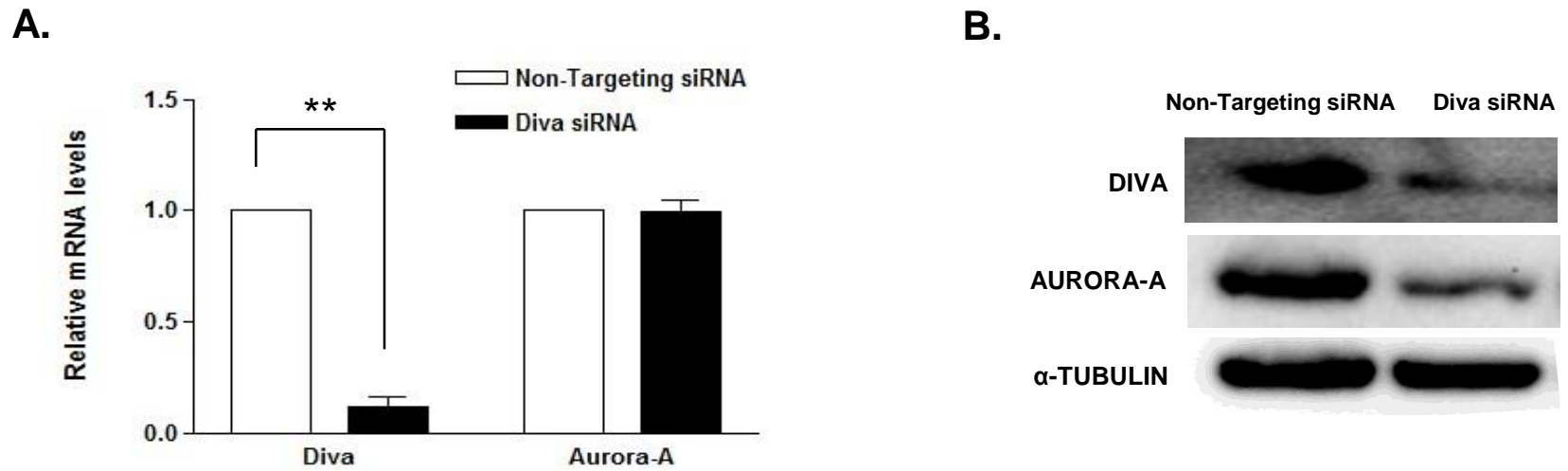




## Result 2. Diva localized at nucleus in cancer cells



# Result 3. Diva regulated translation but not transcription of Aurora A in SKOV3 cancer cells



# Hypothesis (1)

- **Diva has known as a member of Bcl-2 family and has a critical role in apoptosis regulation** (Adams & Cory 1998; Song et al., 1999).
- **It has been reported that Diva, a novel anti-apoptotic member of the Bcl-2 family, blocks apoptosis** (Zhang et al., 2001).

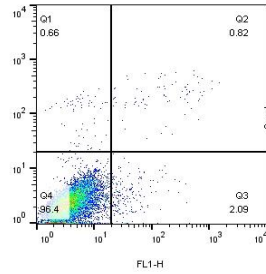
**Hypothesis : Silence of Diva may change apoptosis rate in SKOV3.**



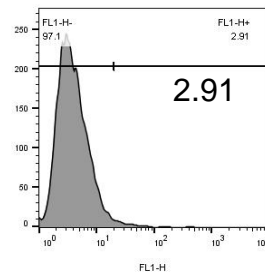
# Result 4. Diva Silencing did not increase apoptosis in SKOV3 cancer cells

**A.**

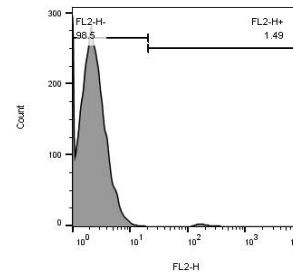
**Non-Targeting siRNA**



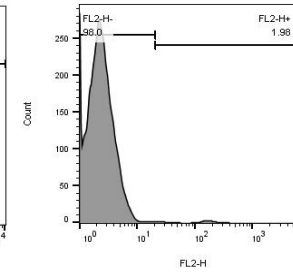
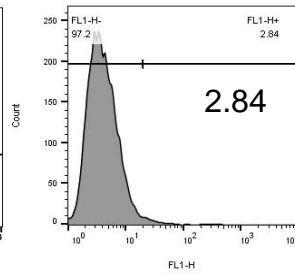
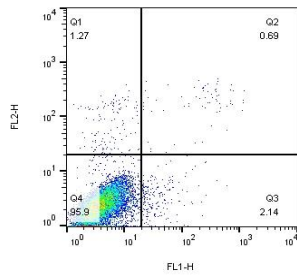
**Annexin V**



**PI**



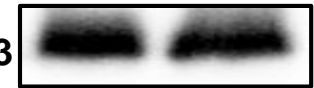
**Divia siRNA**



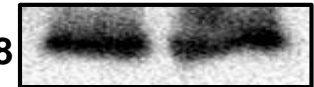
**B.**

**Non-Targeting siRNA    Divia siRNA**

**caspase3**



**caspase8**



**caspase9**



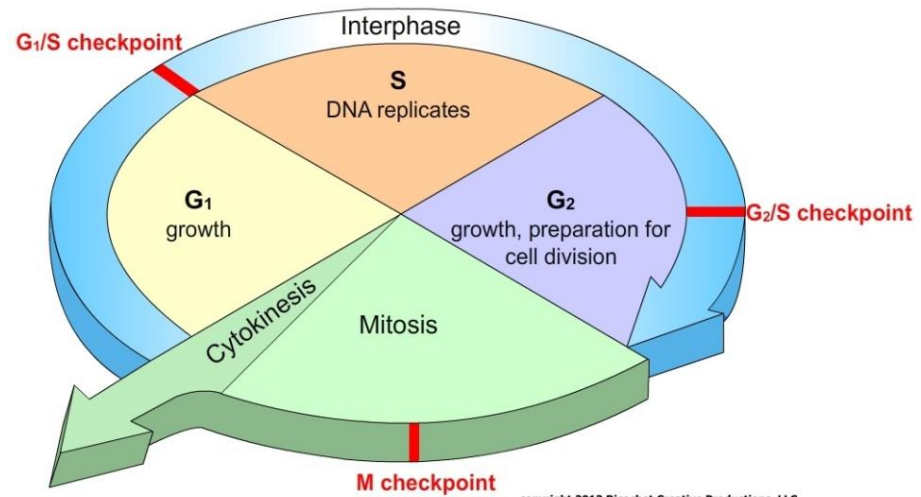
**α-tubulin**



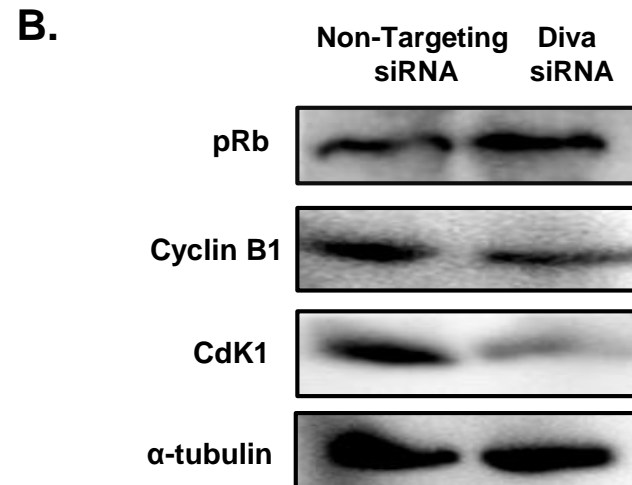
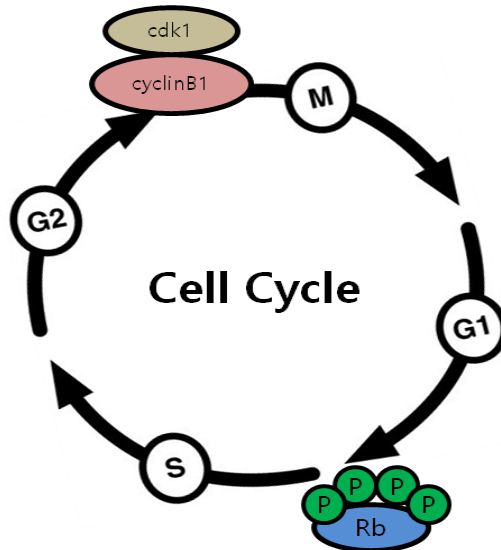
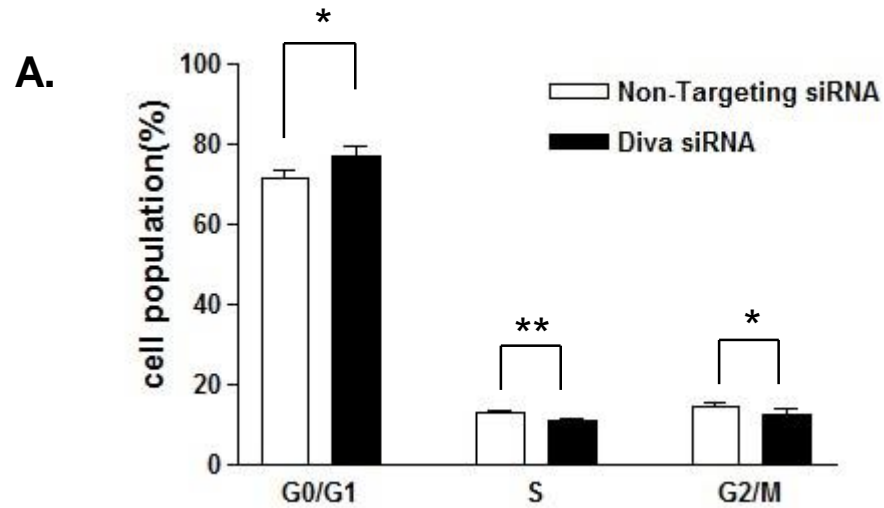
## Hypothesis (2)

- Previous studies have shown that Aurora A may regulate cell cycle progression during the G2-M transition (Ouchi M et al., 2004; He L et al., 2008).

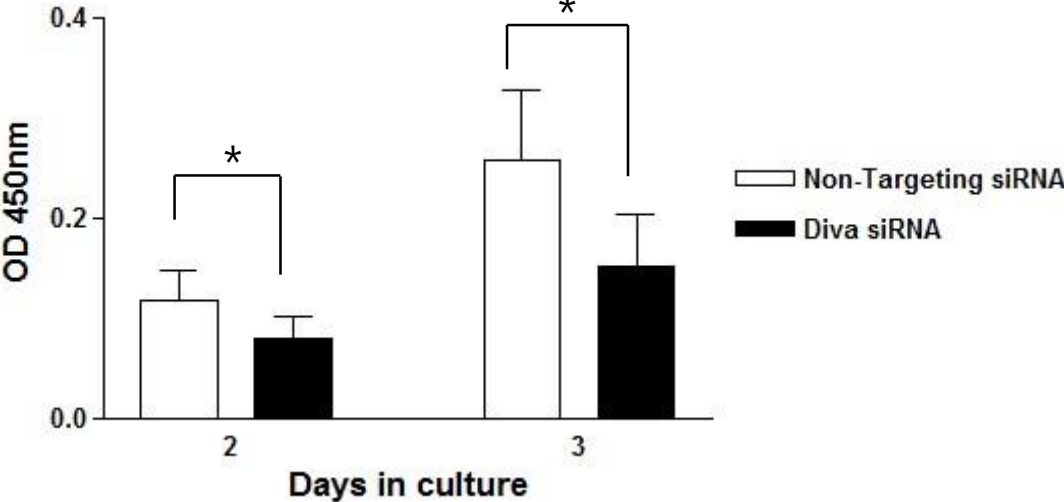
**Hypothesis : Diva knockdown may affect cell cycle in SKOV3.**



# Result 5. Diva silencing reduced cell cycle progression



# Result 6. Diva knockdown reduced cell viability in SKOV3 cancer cells.

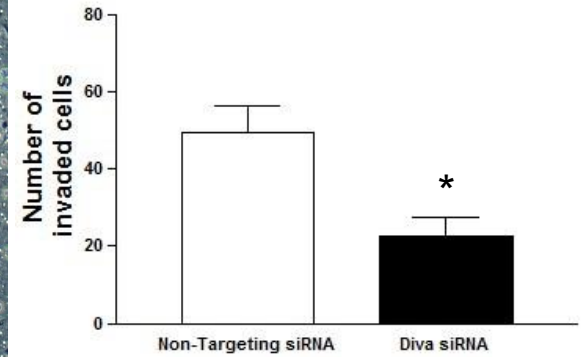
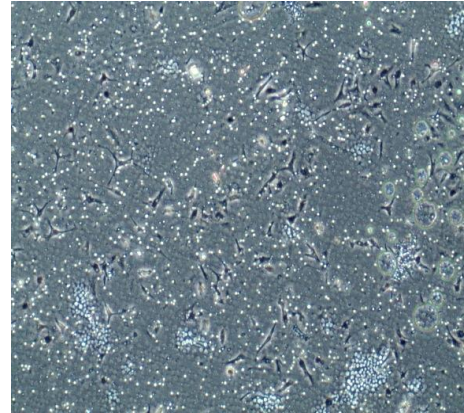
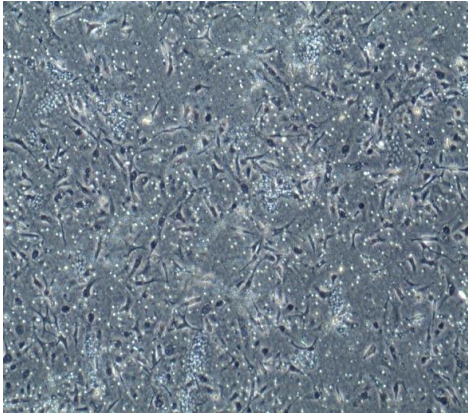


# Result 7. The number of invaded and migrated cells were reduced after Diva knockdown

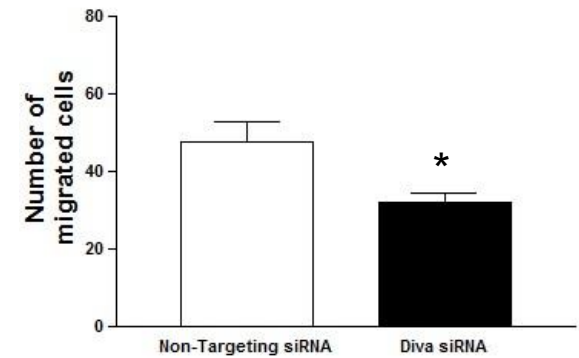
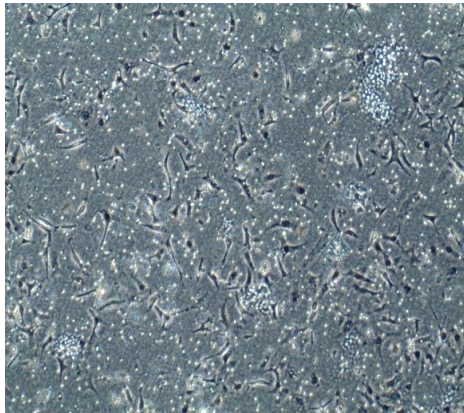
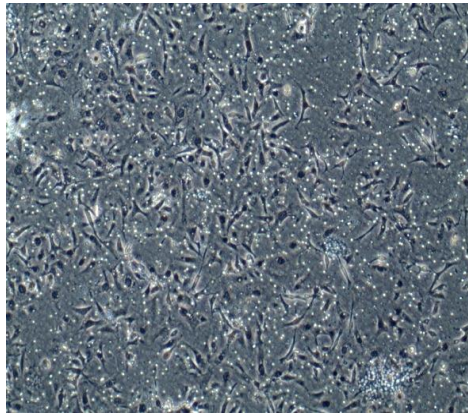
Non-Targeting siRNA

Diva siRNA

Invasion



Migration





# Summary

- **Diva was expressed in a various human cancer tissues.**
- **We firstly confirmed that Diva, as an upstream regulator, regulated translation, but not transcription, of Aurora A in SKOV3 ovarian cancer cells.**
- **Diva knockdown did not affect apoptosis but reduced cell cycle progression in SKOV3 ovarian cancer cells.**
- **Diva knockdown also reduced a viability and the number of invaded and migrated SKOV3 ovarian cancer cells.**

# Conclusions

- **Diva is an upstream regulator of Aurora A in abnormal mitosis of cancer cells as well as in meiosis of oocytes.**
- **Diva knockdown inhibited the various functions of cancer cells, suggesting that Diva may act as an oncogene, and may also be a potential therapeutic target for ovarian cancer.**

# Acknowledgments

# Thank You

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## Lab Members:

- Kyeoung-Hwa Kim, PhD
- Seong-Hee Ko, PhD
- Jinie Kwon, MS
- Eun-Young Kim, MS
- Su Yeon Lee, PhD candidates