Role of Androgen Receptor in Epithelial to Mesenchymal Transition (EMT) in Prostate Cancer Cells

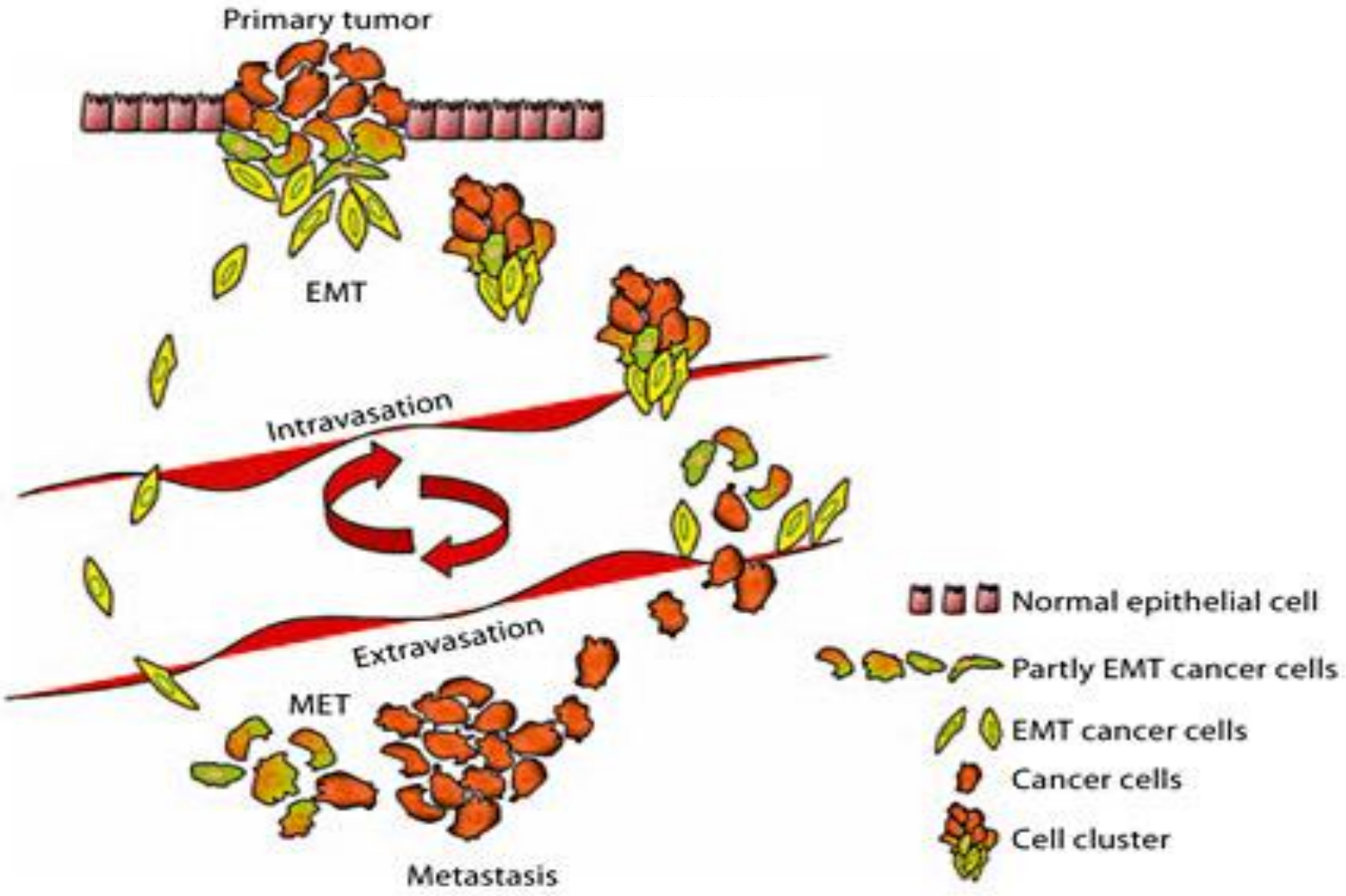
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Challenges in Prostate Cancer Management

- Distinguishing indolent versus aggressive disease
- Treatment of androgen-independent or castration resistant prostate cancer
- Metastasis

Epithelial to Mesenchymal Transition- EMT, a Major Event in Metastasis



Androgenic Stimuli - Facilitator of Inhibitor of EMT?

- **Androgens induce EMT activation in prostate cancer cells (Zhu and Kyprianou, 2010)**
- **Androgen deprivation leads to EMT activation in mouse harboring human PCa xenografts (Sun *et al*., 2012)**
- **Izumi *et al*. (2013) reported EMT activation in prostate cancer cells after AR silencing**

The role of androgenic stimuli in EMT regulation remains to be established

Androgen & its Receptor (AR) as a Regulator of EMT in Prostate Cancer Cells

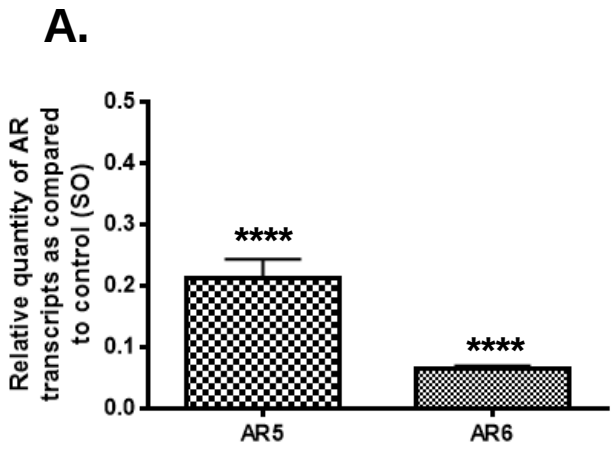
Queries ????

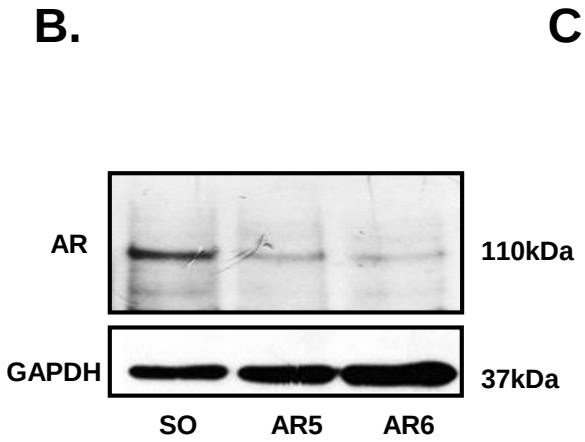
- Whether modulation in the levels of androgen receptor (AR) alters the invasiveness of prostate cancer cells?
- If yes, mechanisms underlying AR driven modulation in invasiveness?

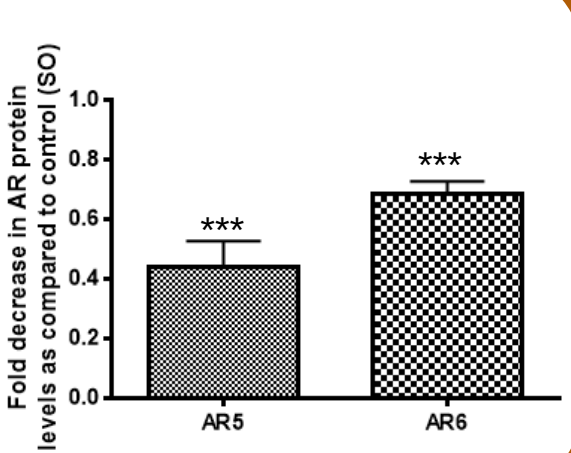
Experimental Models

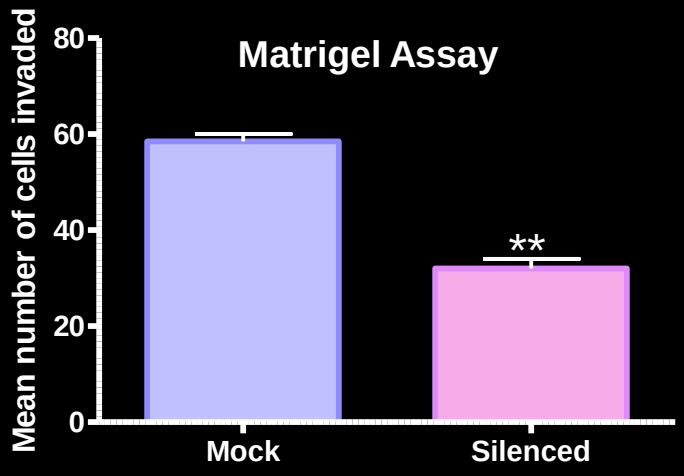
- Androgen dependent prostate cancer cell line (LNCaP-FGC)
- Androgen independent prostate cancer cell lines (PC3 and DU145)

Effect on Invasion of Androgen-Dependent LNCaP cells









AR silencing led to reduction in the invasiveness of androgen-dependent LNCaP cells.

*** p value < 0.001, **** p value < 0.0001

Microarray Analysis to Identify EMT/ Invasion Associated Genes

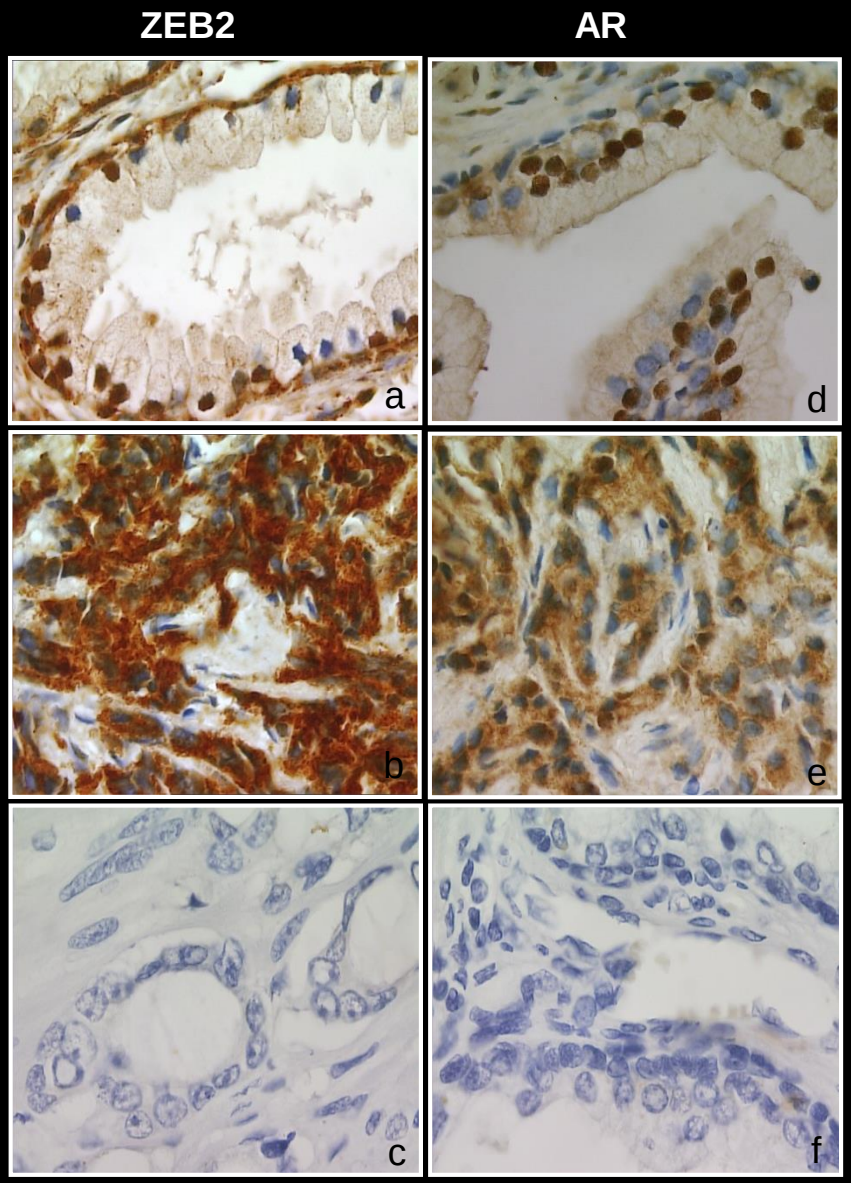
6% differentially expressed genes

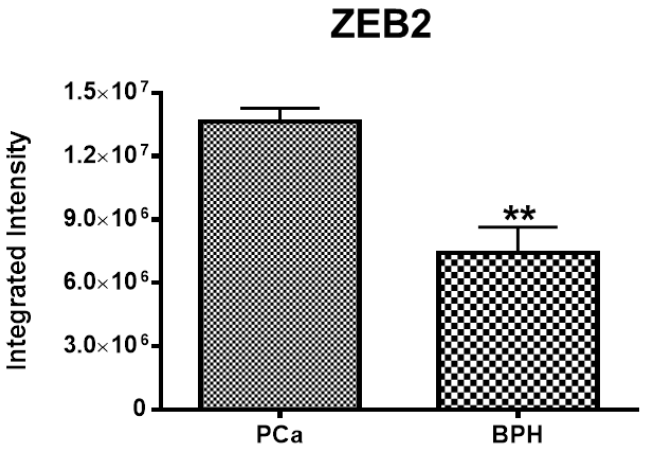
- Twist 2
- ZEB2 (Zinc Finger E Box Binding Homeobox 2) / SIP1
- EVT
- MMP-7
- IGF-1
- Cdc 42

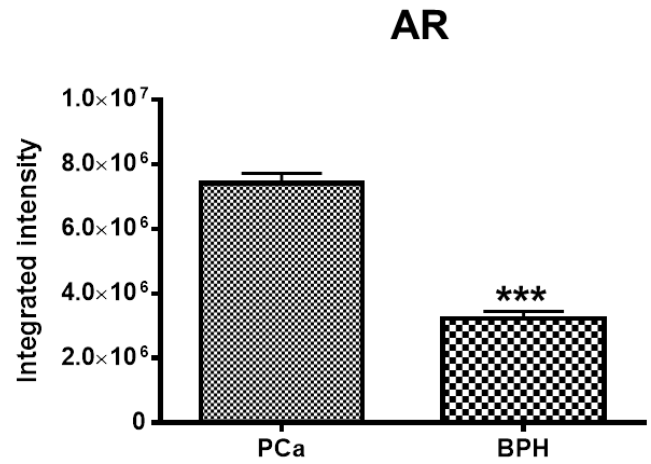
ZEB2 and EMT

- **ZEB2 knockdown suppressed migration and invasion in glioma cells (Qi *et al.*, 2012)**
- **ZEB2 directly binds proximal E boxes within the E-cadherin gene and mediates transcriptional repression by recruiting co-repressor complexes (Postigo *et al*., 2003)**

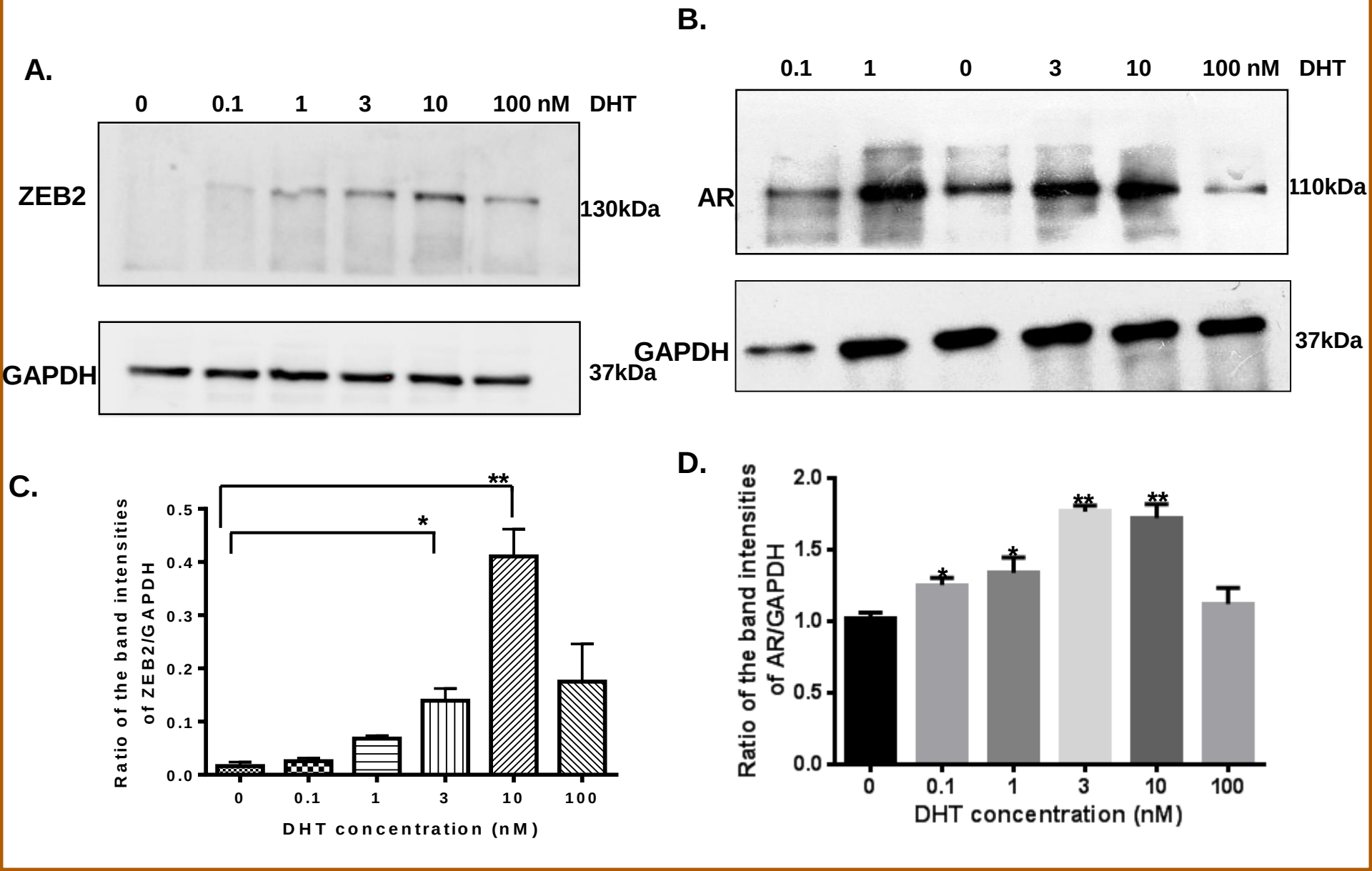
AR and ZEB2 Expression in Prostate Cancer and Benign Prostatic Hyperplasia



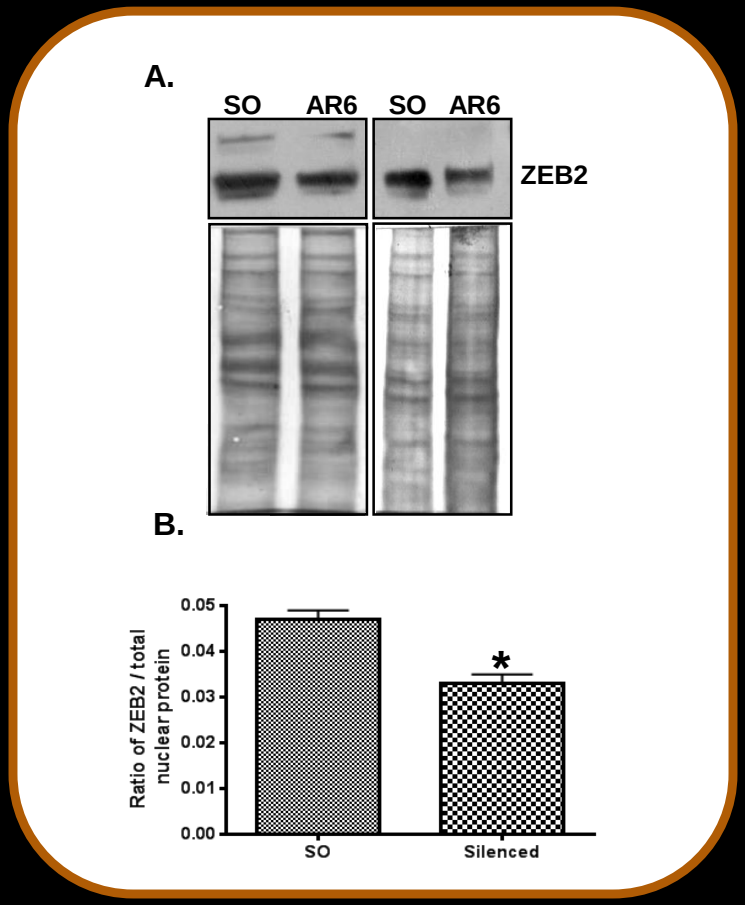


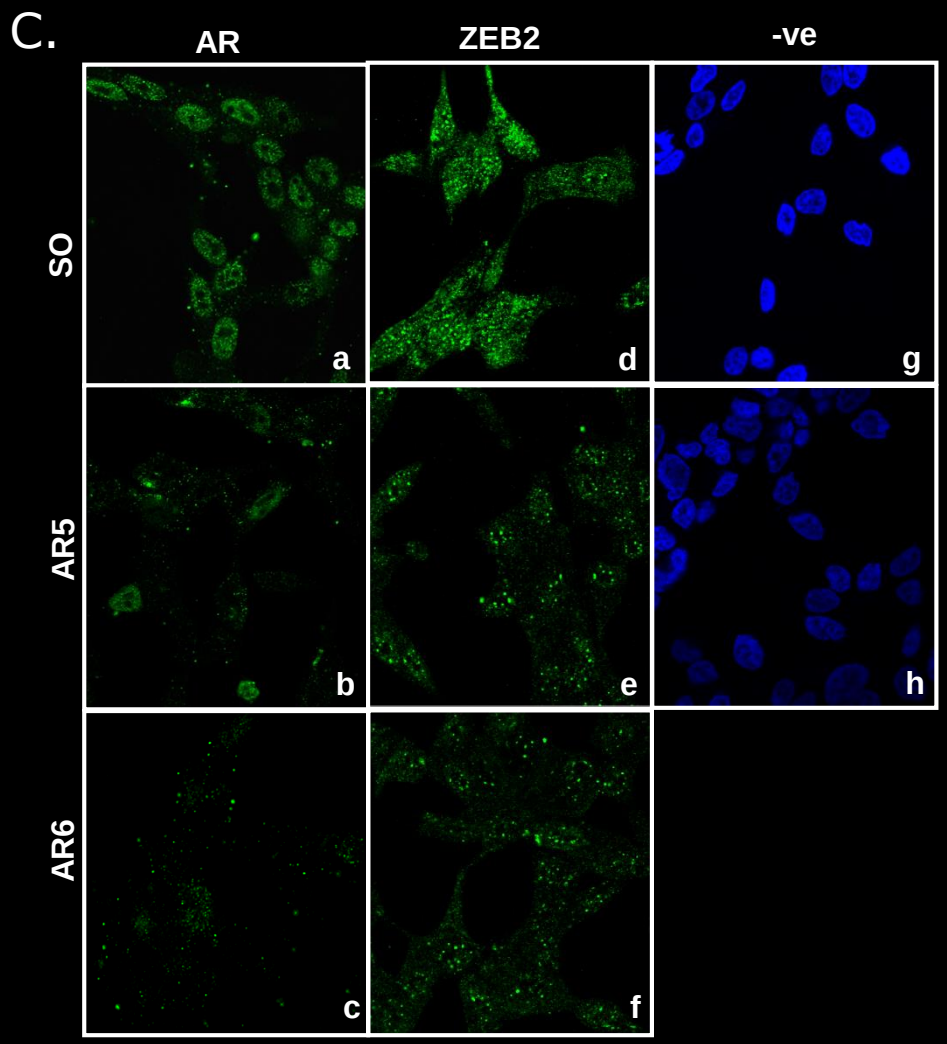


Androgen stimulates ZEB2 expression in androgen-dependent LNCaP cells



ZEB2 expression in AR silenced LNCaP cells



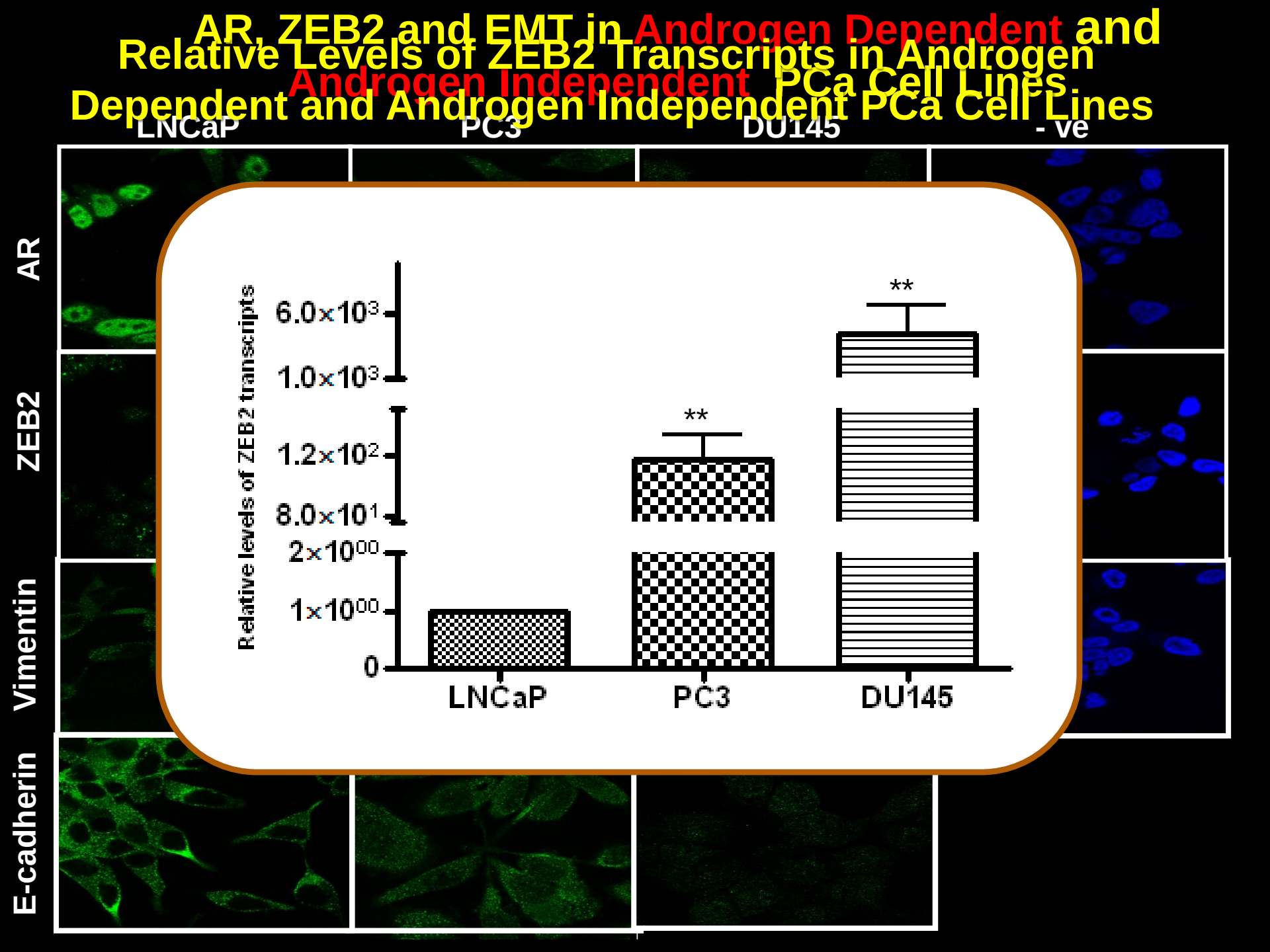


AR positively regulates ZEB2 expression in androgen-dependent LNCaP cells

Whether the levels of ZEB2 differ in androgen dependent (less metastatic) and androgen independent (more metastatic) prostate cancer cell lines?

AR, ZEB2 and EMT in Androgen Dependent and Androgen Independent PCa Cell Lines

Relative Levels of ZEB2 Transcripts in Androgen Dependent and Androgen Independent PCa Cell Lines

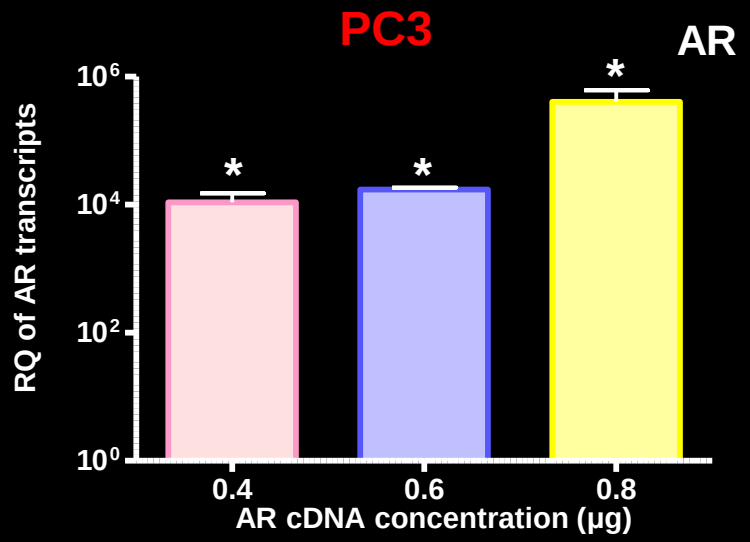


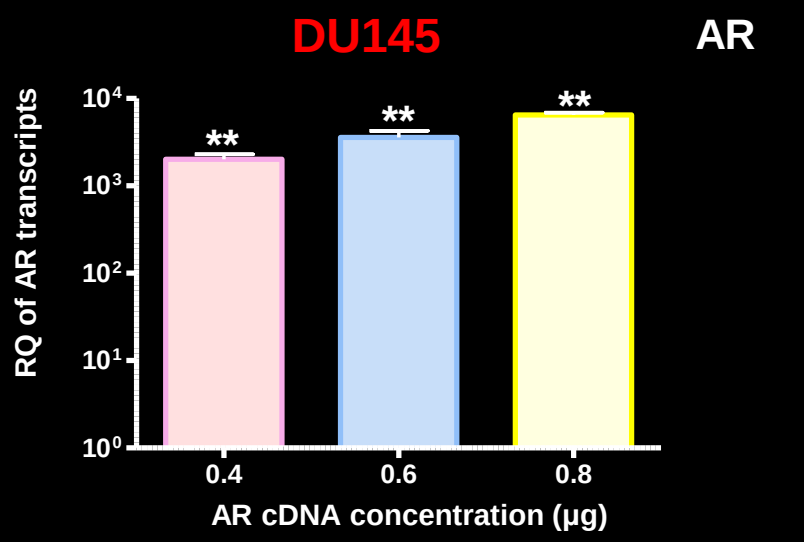
AR over-expression in androgen independent prostate cancer cells

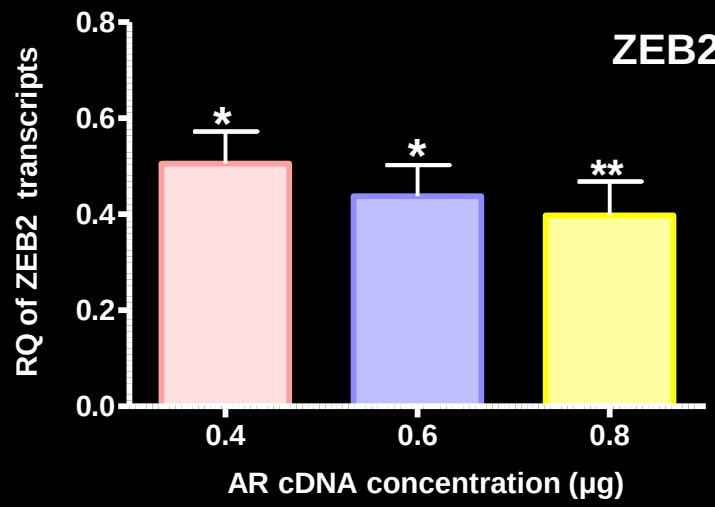


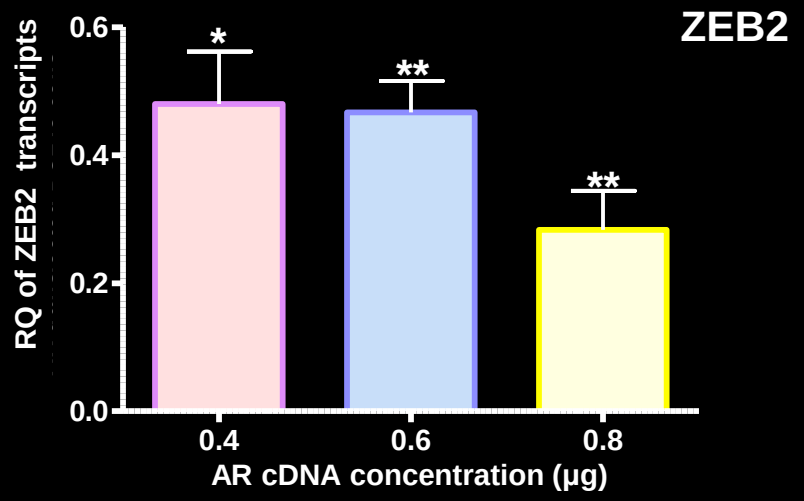
ZEB2 levels?

Transcript Levels of AR and ZEB2 in AR transfected PC3 and DU145 Cells





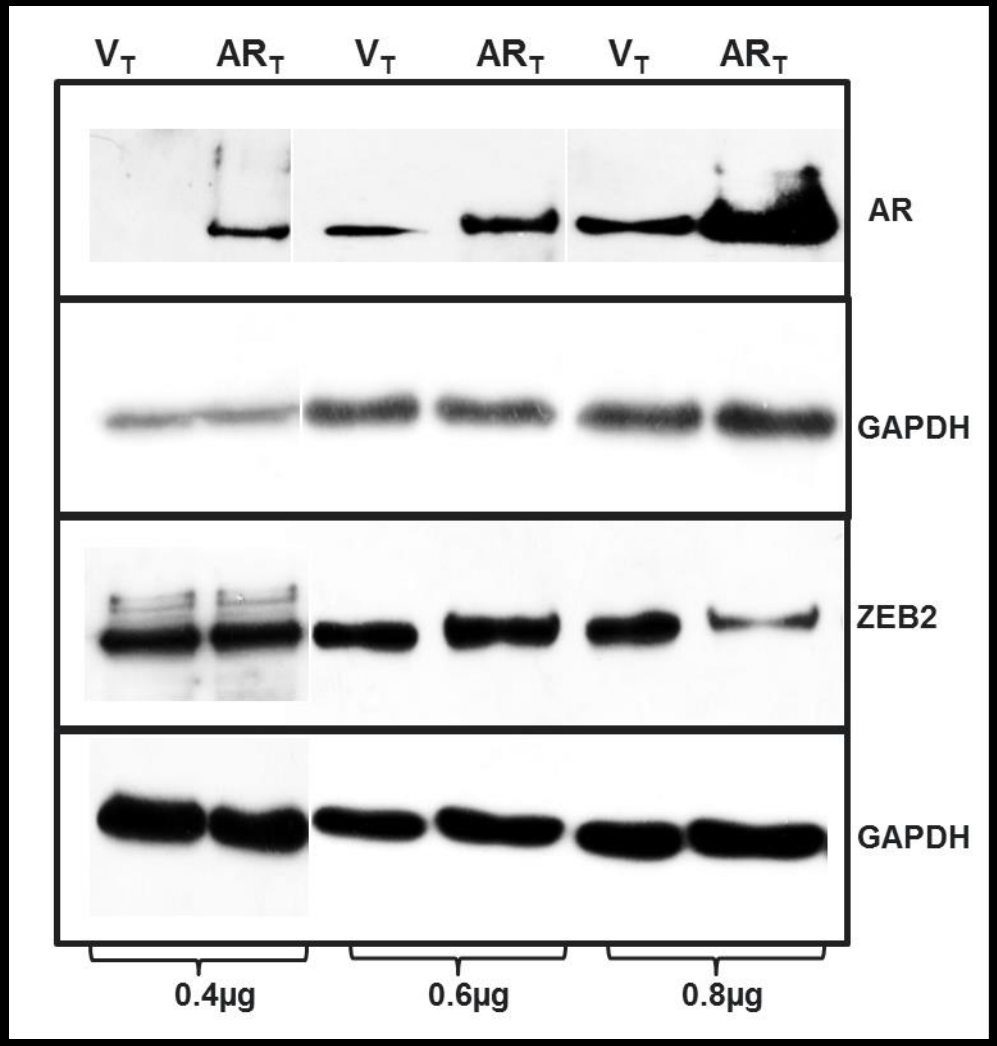


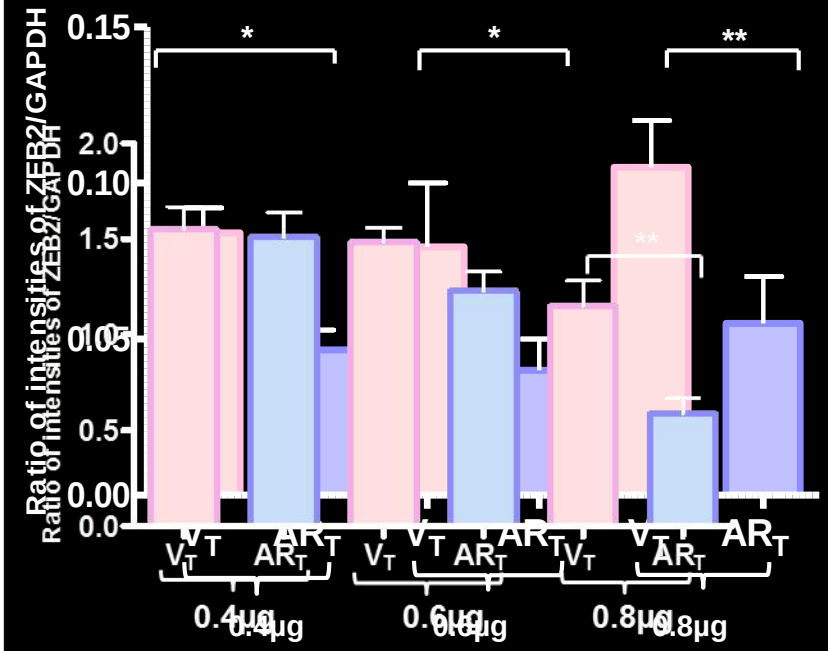


AR over-expression led to a decrease in ZEB2 transcript levels.

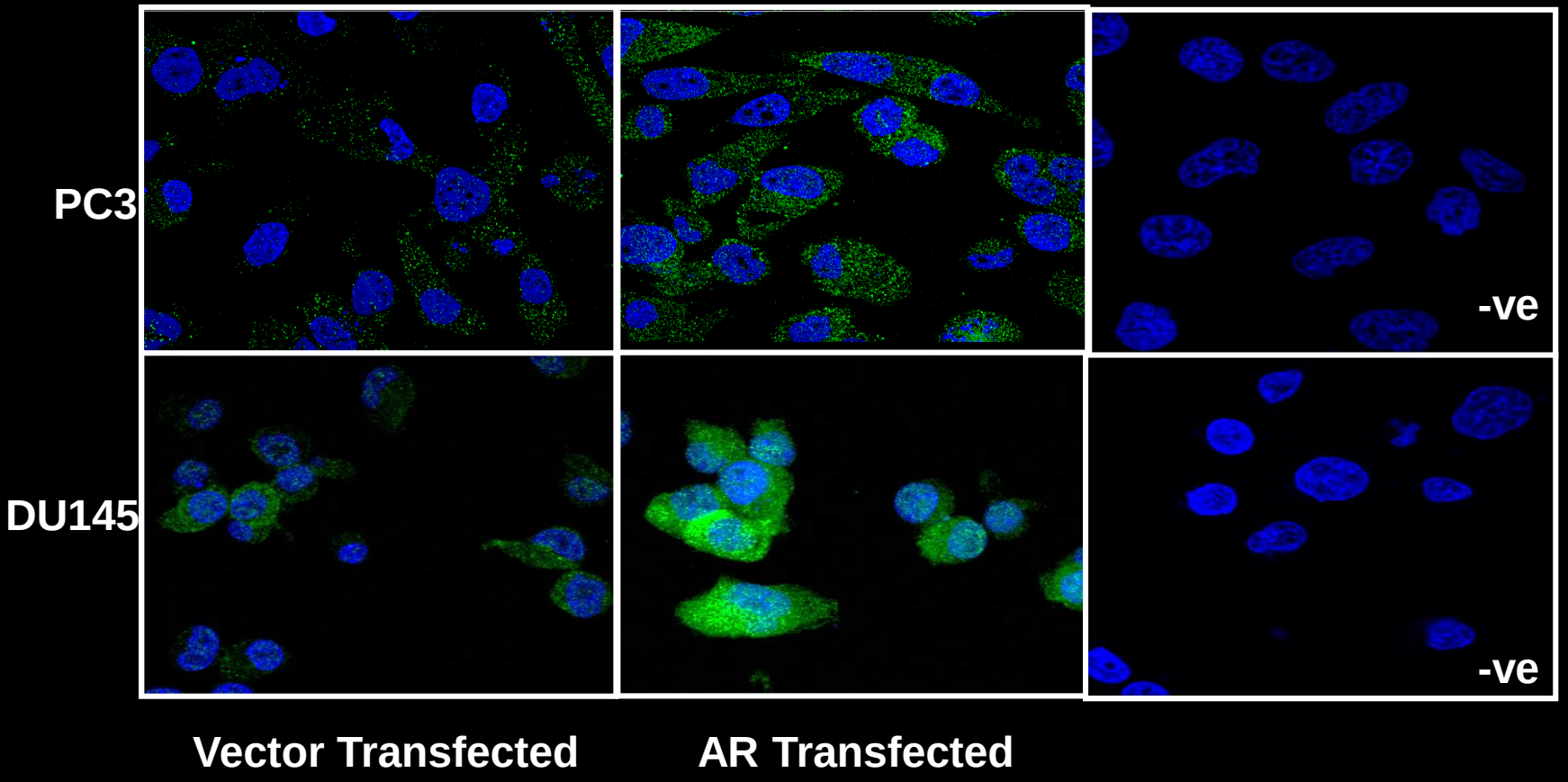
ZEB2 Protein Levels in AR Over-expressing Cells

DU145

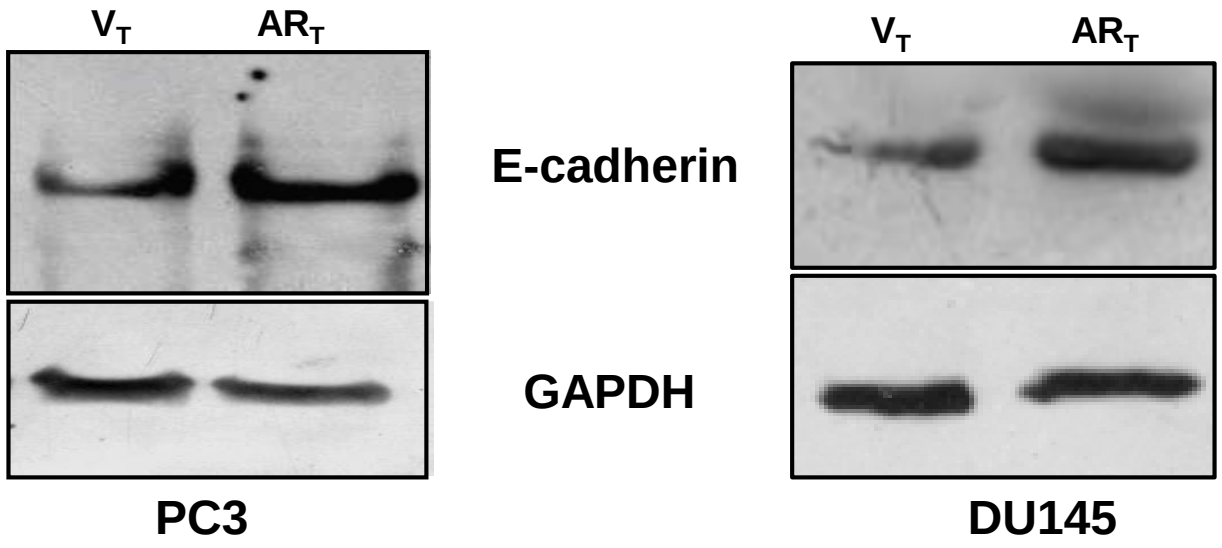




Expression of E-cadherin in AR Over-expressing Androgen Independent Prostate Cancer Cells

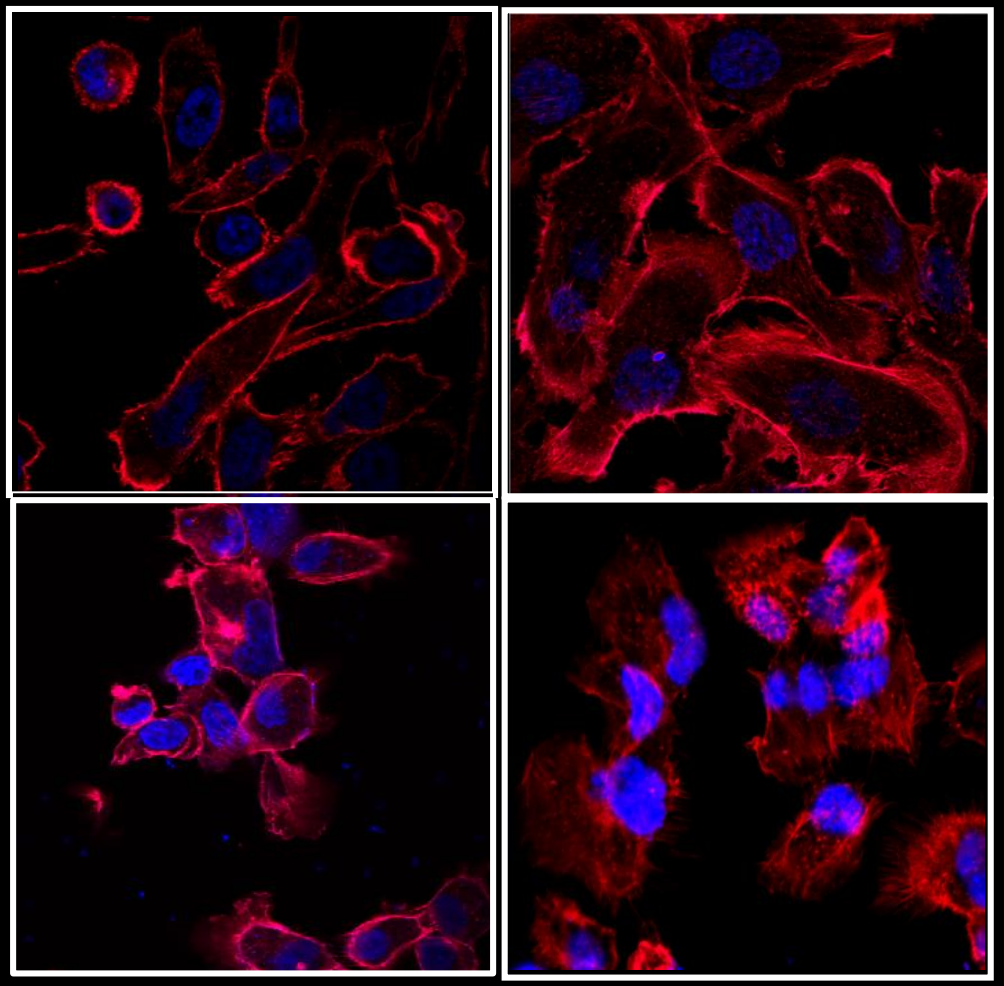


Expression of E-cadherin in AR Overexpressing Androgen Independent Prostate Cancer Cells

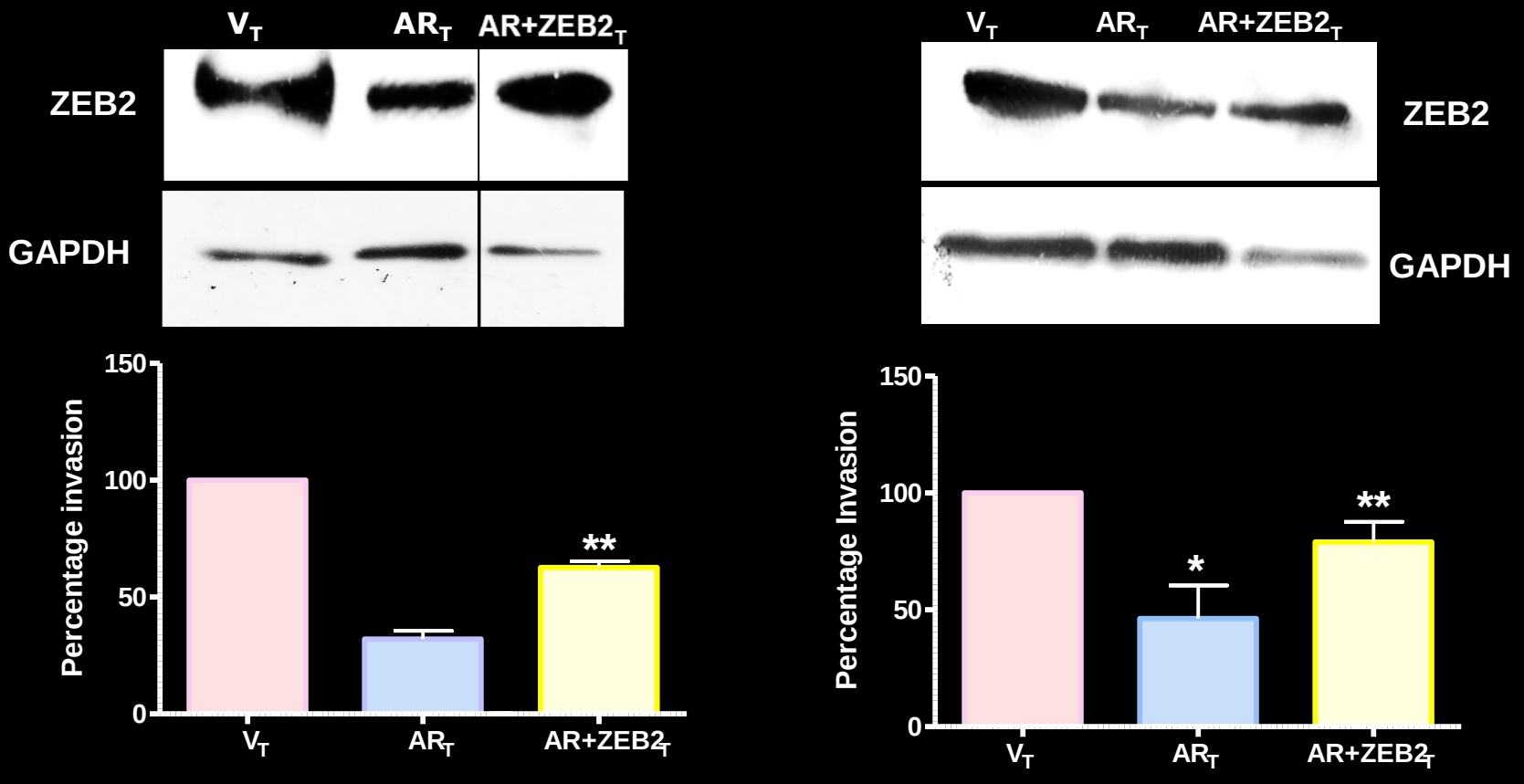


E-cadherin expression is increased following AR over-expression.

Morphology of AR Over-expressing Androgen-independent Prostate Cancer Cells

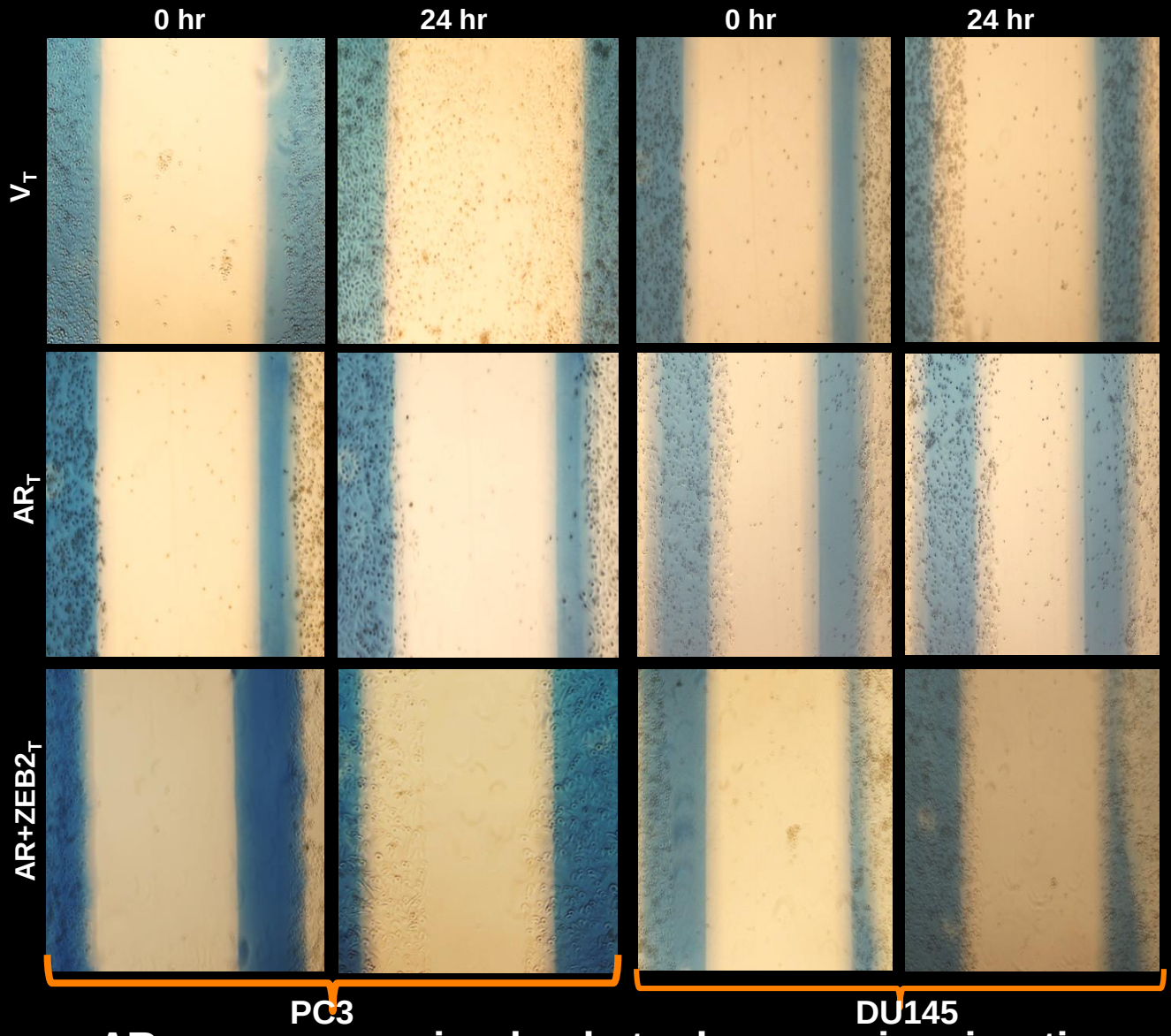


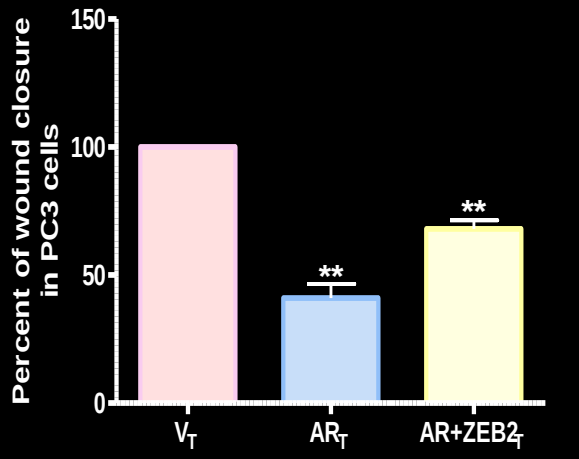
Invasiveness of AR Expressing Androgen Independent Cells and Rescue by ZEB2 Over-expression

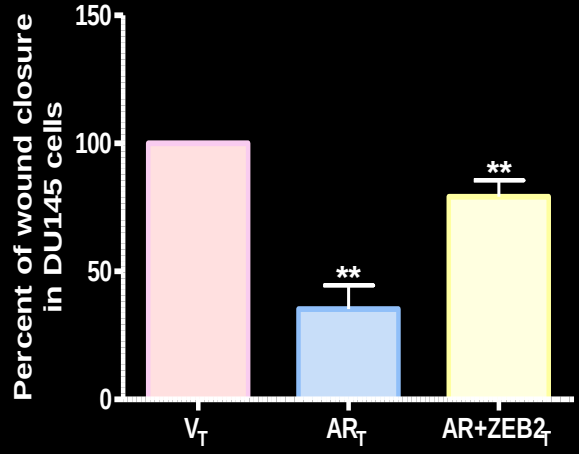


ZEB2 over-expression restores invasion in AR over-expressing androgen independent prostate cancer cell lines

Migration of AR Over-expressing Androgen Independent PCa Cells and Restoration by ZEB2 Over-expression





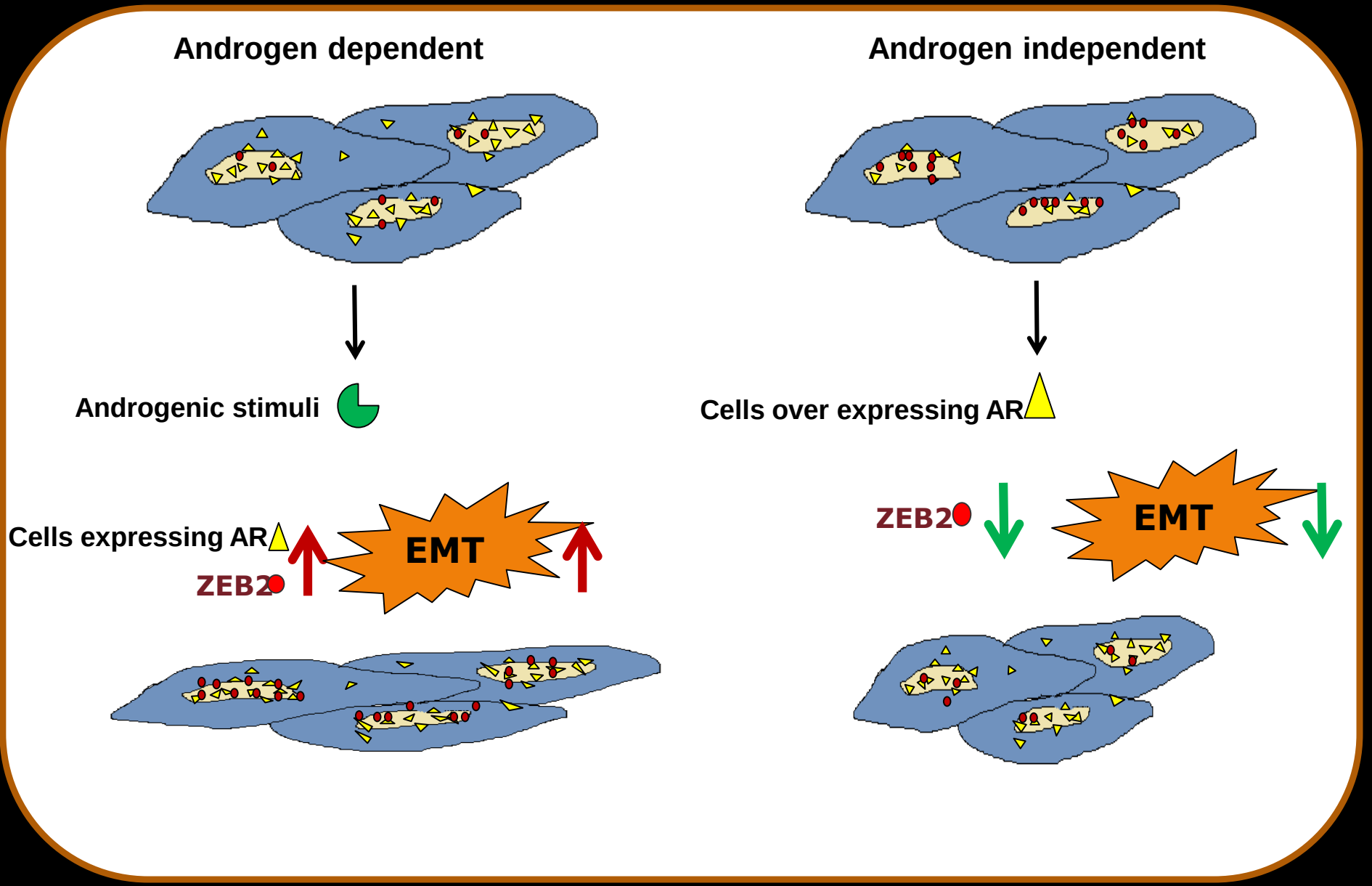


AR over-expression leads to decrease in migration and ectopic expression of ZEB2 restores the migration of AR over-expressing cells.

Conclusion

- AR negatively regulates ZEB2 expression in androgen independent prostate cancer cells
- Expression of E-cadherin is increased in AR over-expressing prostate cancer cells
- Invasion and migration are adversely affected, following AR over-expression, in androgen independent prostate cancer cells

To summarize…



Take Home message...

Intermittent, rather than continuous androgen deprivation therapy is more beneficial to patients with locally advanced metastatic prostate tumors.

Acknowledgement

- Dr. Geetanjali Sachdeva, Ph. D
- Mr. Sumeet Nayak (graduate student)

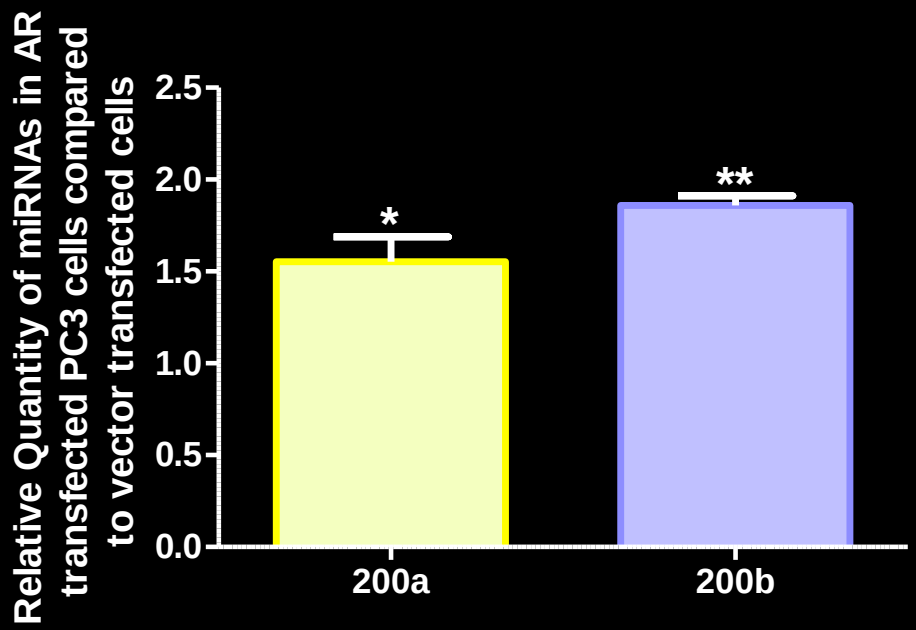
- Dr. Donald Tindall, Mayo Clinic, US
- Dr. Danny Huylebroeck, University of Leuven, Belgium

- Indian Council of Medical Research (ICMR)
- Department of Atomic Energy-BRNS

In Silico Scanning for the Presence of ZEB2 Binding Sites in the Regulatory Regions of miR200a and 200b Genes

Gene name	Regions Scanned	E box-2 CACCTG	Z box -1 CAGGTG	Z box-2 CAGGTA
miRNA 200a	1102243-1103242	-	-941, -418, -364	-
miRNA 200b	1101484-1102483	-	-938, -494, -182	-

miR200a and 200b Levels in AR Over-expressing PC3 Cells



miR200a and miR200b levels are increased in AR over-expressing prostate cancer cells.