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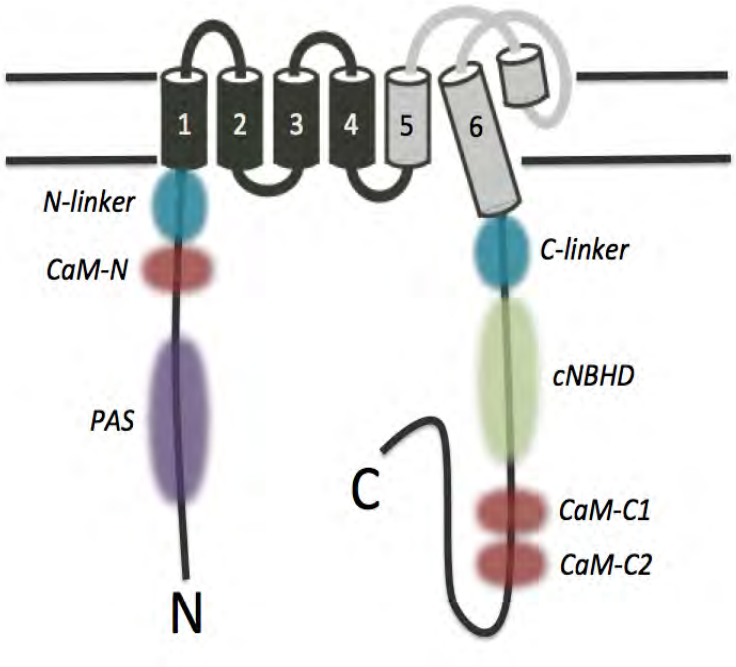
Calcitriol and Retinoic Acid Downregulates Human Ether à-go-go Potassium Channel in SiHa Cervical Cancer Cells Via a Canonical Pathway

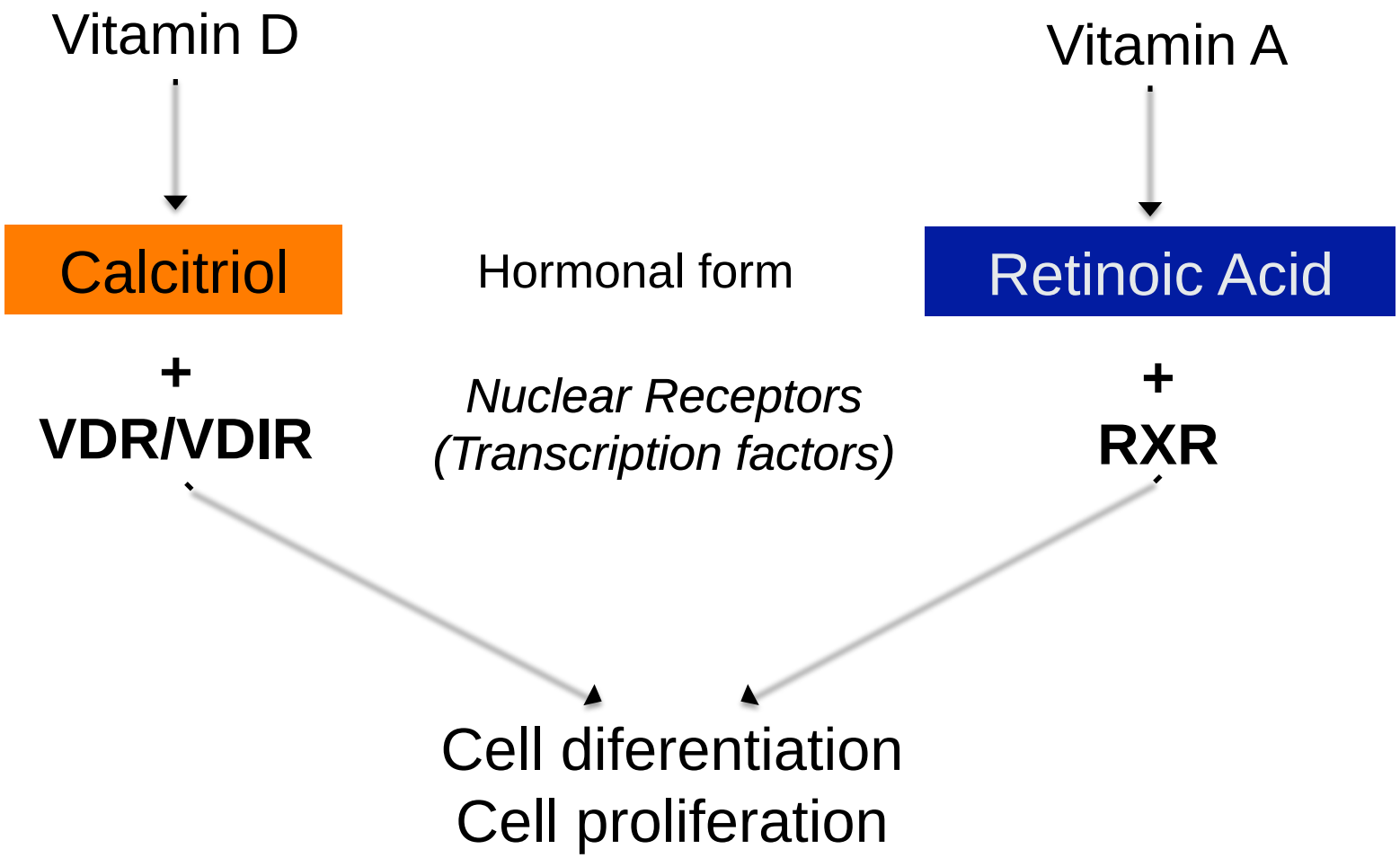
Ether à-go-go potassium channel EAG1 is also an oncogene



Structure of the one α subunit

- ✓ EAG1 is normally expressed in brain and CNS.
- ✓ EAG1 function in brain and CNS in unknown.
- ✓ EAG1 expression in other tissues is related to cancer development and progression.
- ✓ EAG1 is considered an oncogene.
- ✓ EAG1 is an interesting therapeutic target in oncology.

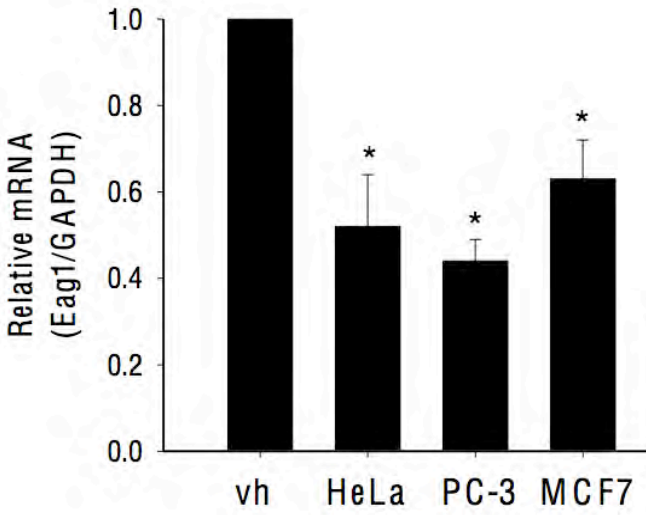
Vitamins with hormonal actions are also anticancer molecules

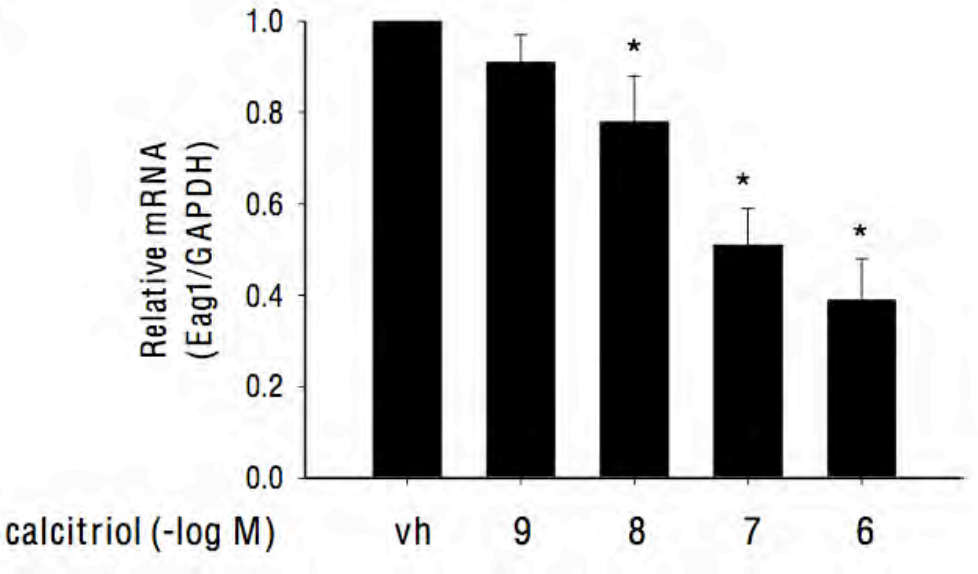


Nuclear receptors bind to response elements in their target genes

- ✓ Example: TRPV6 ion channel expression is induced by calcitriol via VDR through a VDRE in the promoter region.

Our group reported EAG1 downregulation by calcitriol in cancer cell lines





Avila *et al*., Anticancer Res. 2010

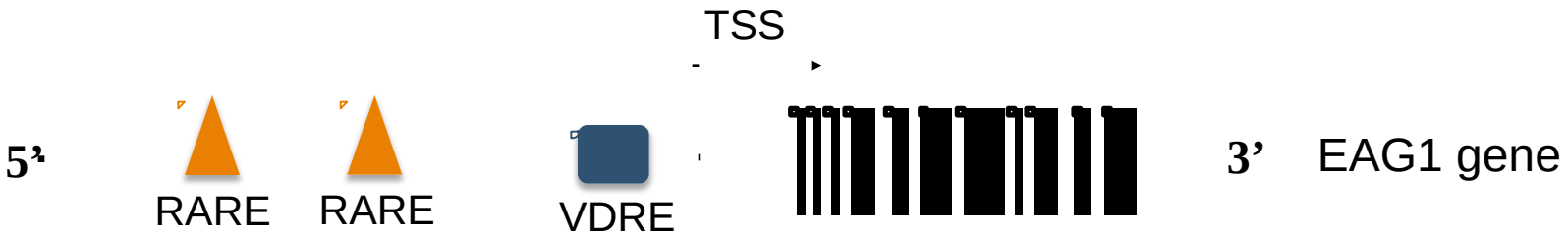
- ✓ The mechanism was unknown.
- ✓ My PhD project has been to study the mechanism.
- ✓ We also evaluated retinoic acid because has anticancer effects.

Calcitriol down-regulates EAG1 expression in a cervical cancer cell line (SiHa)

- ✓ We used the SiHa cell line because express high levels of EAG1.
- ✓ Immunohistochemistry in SiHa cells treated with calcitriol or retinoic acid showed lower EAG1 protein compared to vehicle treated cells.
- ✓ RT-qPCR showed that mRNA is also reduced in calcitriol or retinoic acid treated SiHa cells, this result is agree with our previous reports.

In silico analysis: EAG1 gene has putative response elements to calcitriol (VDRE) and retinoic acid (RARE)

- ✓ Computational analysis using MatInspector program revealed putative response elements in the EAG1 gene promoter.



EAG1 gene has putative response elements to calcitriol (VDRE) and to retinoic acid (RARE)

- ✓ To explore the regulatory mechanism of calcitriol or retinoic acid on hEAG1 transcription repression we cloned the EAG1 gene promoter into a reporter vector.

- ✓ Dual-Luciferase reporter system showed that calcitriol and retinoic acid reduced the EAG1 gene promoter activity.

- ✓ To investigate the binding activity of the putative VDRE's and RARE's *in vitro*, we carried out electrophoretic mobility shift assays. We found one VDRE and one RARE into the EAG1 gene promoter.

- ✓ To confirm the binding activity of the putative VDRE's and RARE's *in vivo,* we carried out chromatin immunoprecipitation assays. We found that the VDR binds to VDRE and the RXR binds to RARE.

Summary

- ✓ EAG1 gene promoter has one negative response element to vitamin D receptor and one negative response element to retinoid X receptor.
- ✓ This work provides mechanistic insights on EAG1 oncogene repression by calcitriol and retinoic acid in cervical cancer cells.

Cázares *et al*., under review by NRC Biochimestry and Cell Biology

Concluding remarks

- ✓ Vitamin D and vitamin A are natural compounds with anticancer activity.
- ✓ Endogenous levels are important to prevent cancer.
- ✓ There are clinical studies that evaluate therapeutic doses for cancer patients.
- ✓ Synthetic analogs of those molecules are developed by pharmaceutical companies because of their potential therapeutic use.

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