

# Anti-tumoral activity of Acetogenins derived from Soursop Fruit (*Annona muricata* L.) in High Grade Serous Ovarian Cancer

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## Background

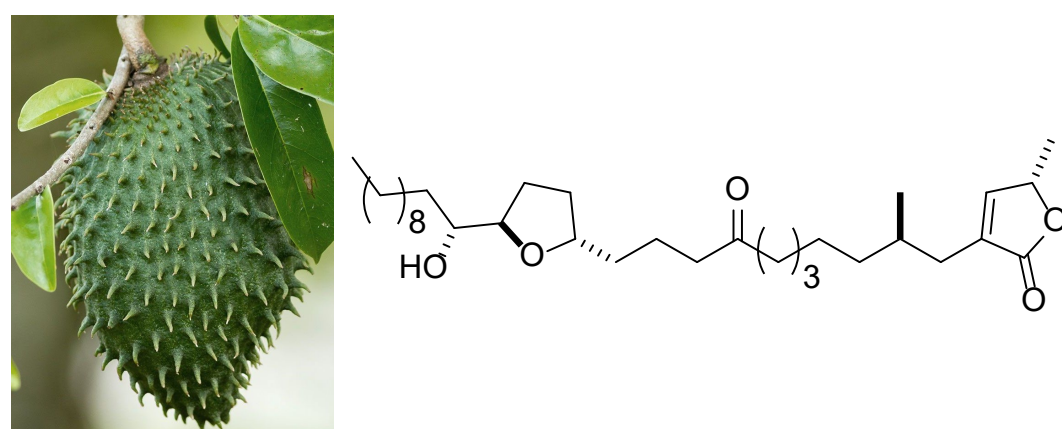
In 2021, the American Cancer Society estimated that approximately 21,410 women will receive a new diagnosis of ovarian cancer and about 13,770 women will die from ovarian cancer in the United States. This type of cancer is treated with cyto-reductive surgery, radiotherapy and hormone therapy, but unfortunately, these treatments are often insufficient, and patients frequently relapse and succumb to the disease (Yajid et al., 2018).

The *Annona muricata* plant contains specific compounds denominated acetogenins (ACGs), which are characterized by C35 or C37 unbranched fatty acid with a  $\gamma$ -lactone at the end of the skeleton (Kim et al., 1998). A single report demonstrated ACGs might increase or decrease the expression of Notch receptors (Li et al., 2017) in cancer cells. The Notch signaling pathway controls a variety of cellular processes, such as cell proliferation, differentiation, apoptosis, and survival. Additionally, abnormalities of Notch signaling is associated with oncogenesis, which usually manifests as abnormality ligands, receptors, and downstream proteins (Hu et al., 2012).

In this study, we isolated and characterized ACGs, modulators of the Notch pathway, and demonstrate their antitumoral activity in ovarian cancer.

## General Aims

Our primary goals were to characterize the Notch signaling-pathway and explain the anti-tumoral activity of ACGs in ovarian cancer. We hypothesized there would be a down-regulation of the Notch receptors as well as reduced metastatic capability following treatment with ACGs.



**Figure 1.** Soursop fruit (left) and structure of Annonacin-10-ona (right).

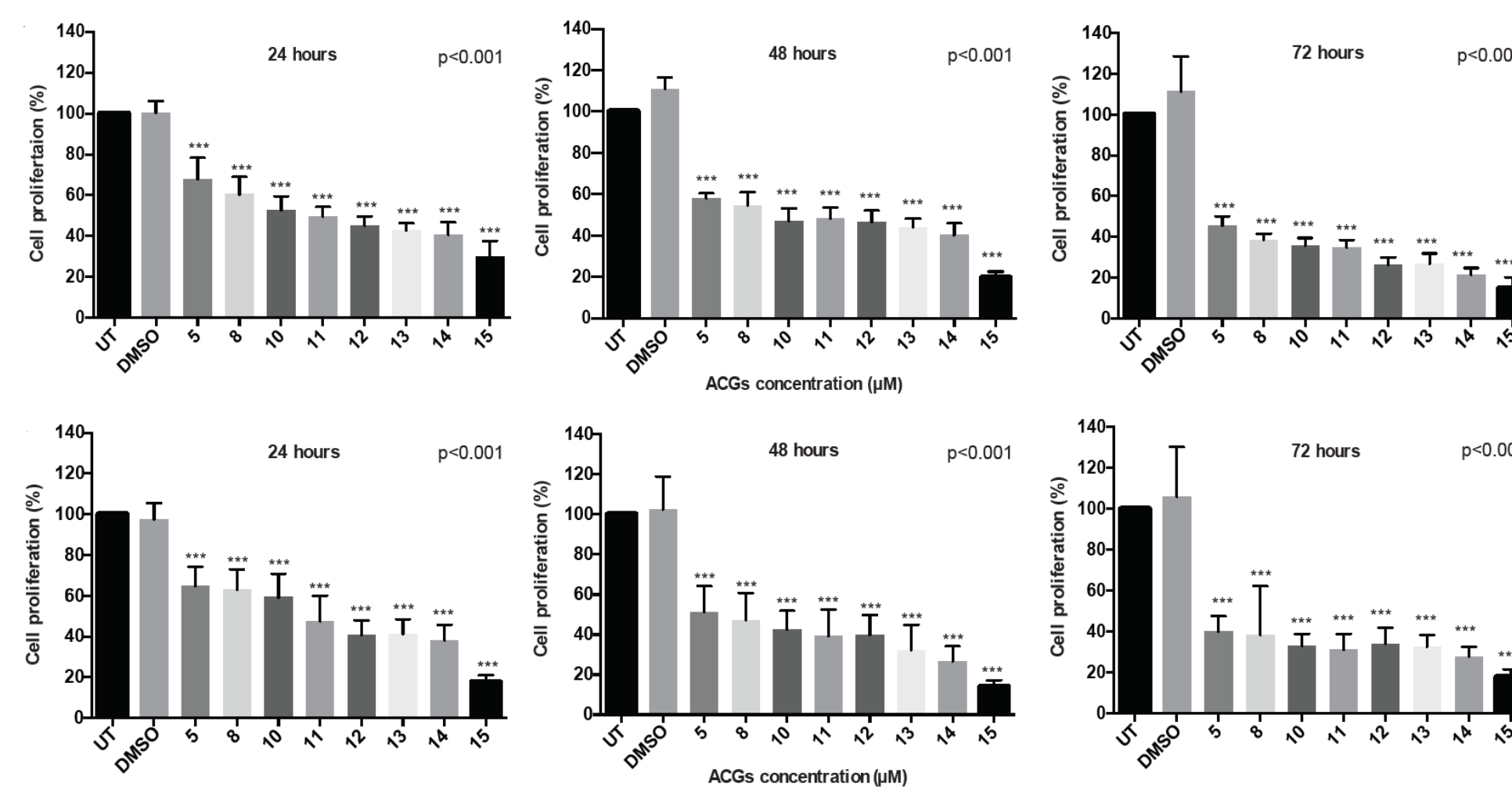
## Methods

Cells were plated onto 24-well plates (300 cells/well). After, they were treated with ACGs (0, 15, and 25 nM). They were incubated in 5% CO<sub>2</sub> and 95% air at 37 °C for 10 days to form colonies. At the end of the incubation, the colonies were stained with crystal violet and counted.

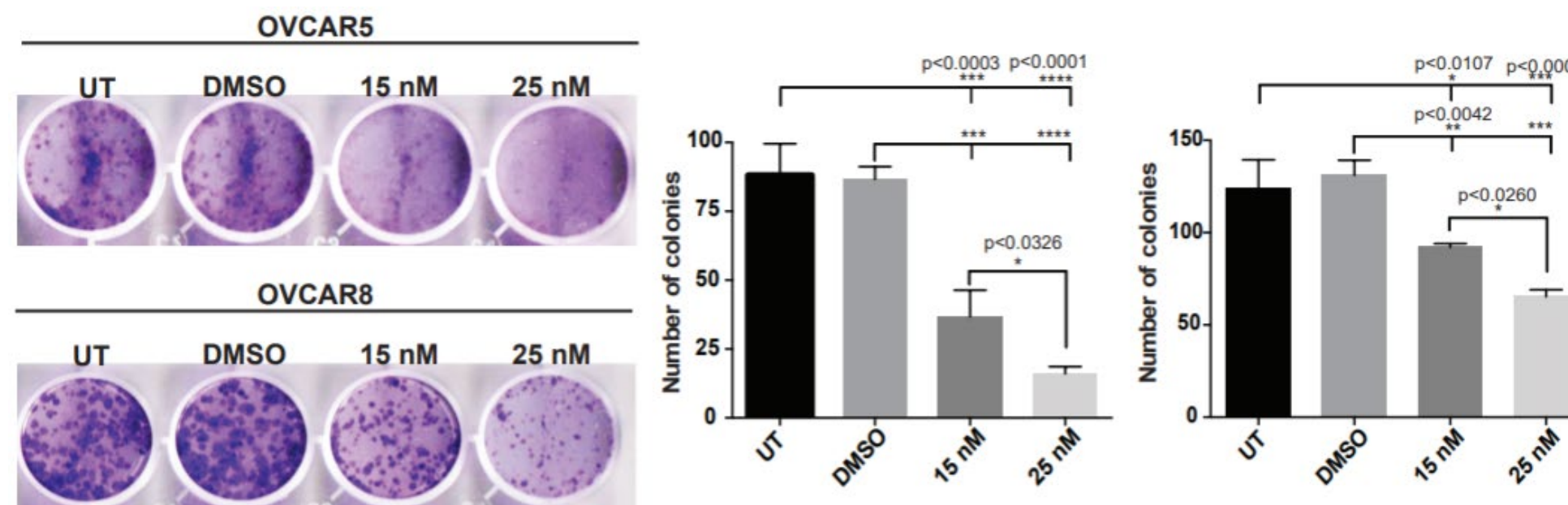
To discover the effects of ACGs on cell proliferation, we performed an Alamar-Blue assay with OVCAR5 and OVCAR8 cells at 24, 48, and 72 hours.

Wound-healing assay was performed by first seeding the cells onto a 6-well plate, scraping the surface, and monitoring for 24 hours as the area closed.

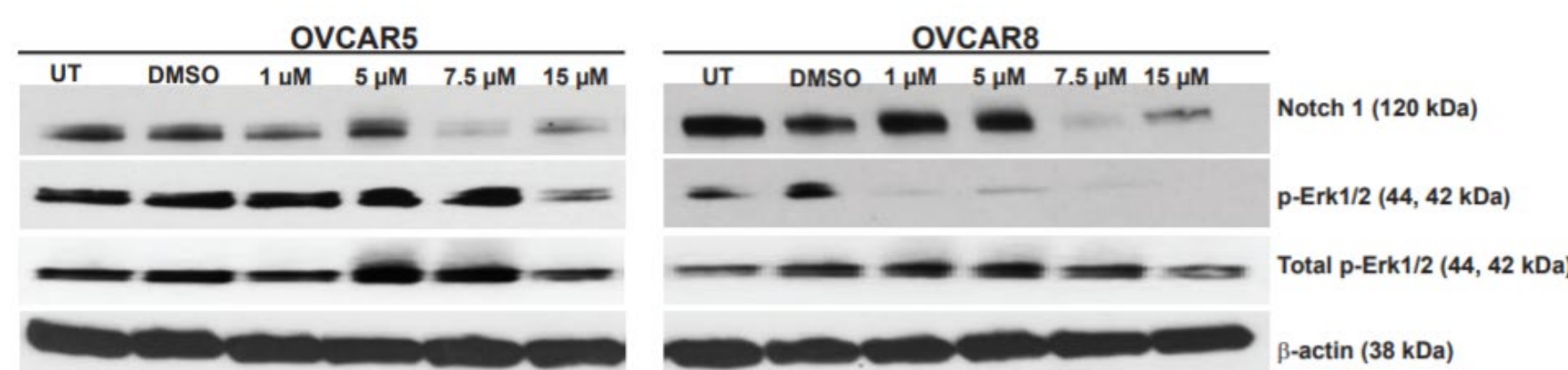
## Results



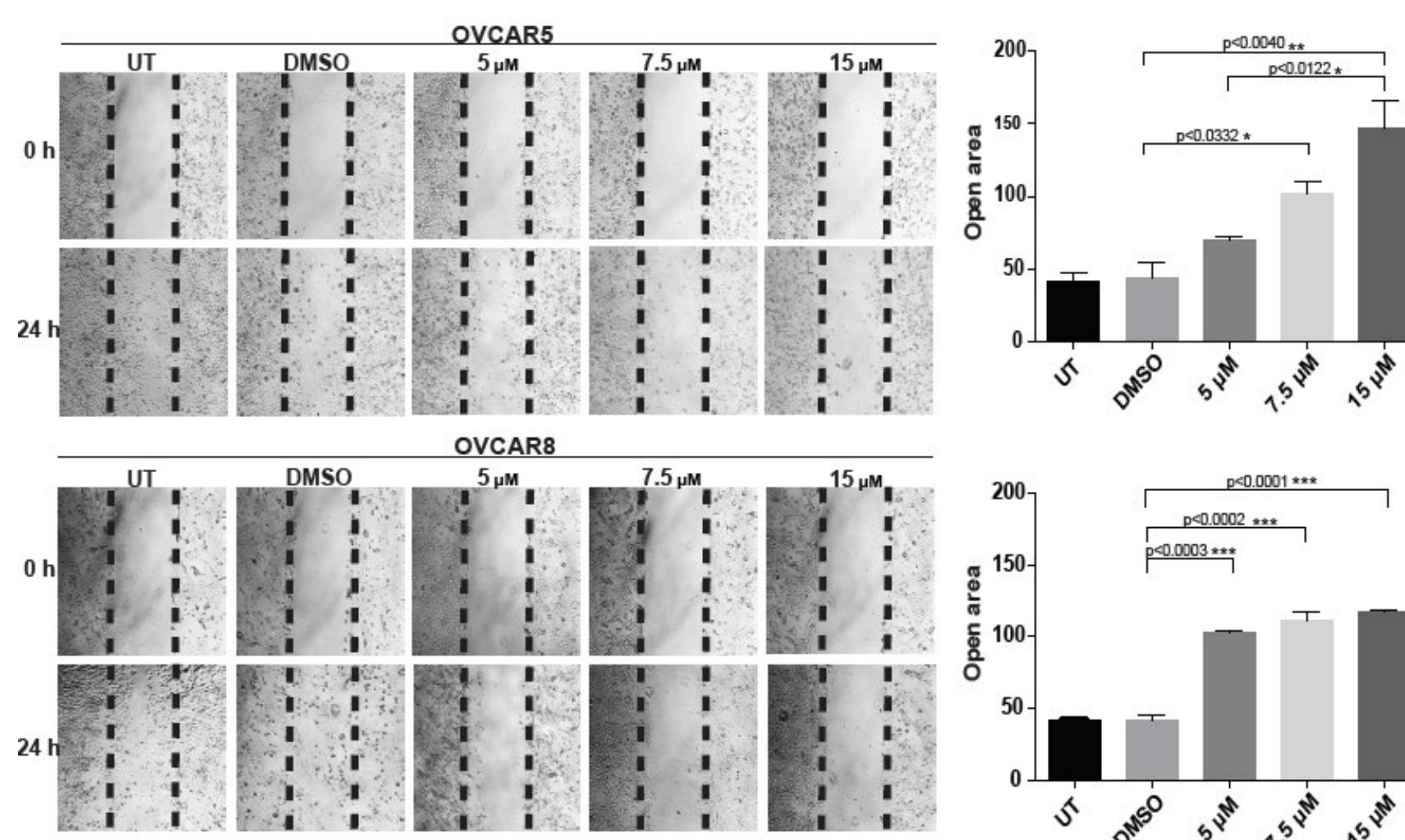
**Figure 2.** ACGs were found to inhibit proliferation of OVCAR5 (top) and OVCAR8 (bottom) cells *in vitro*.



**Figure 3.** It was observed that ACGs significantly decreased colony formation in both OVCAR5 ( $p=0.004$ ) and OVCAR8 ( $p=0.0042$ ) cells compared to the negative controls.



**Figure 4.** Western blot analysis showed a reduction of Notch1 and p-Erk1/2, in line with the decreased colony formation observed.



**Figure 5.** Wound-healing assay showed a reduction of migratory capacity following treatment with ACGs.

## Conclusions

In this study, we demonstrated that using ACGs to target Notch signaling has both antitumoral and anti-metastatic effect in high grade ovarian cancer. The many biological and clinical aspects of ovarian tumorigenesis indicate that an aberrant activation of Notch signaling pathway is involved in tumor initiation, progression, metastasis, resistance to chemotherapy, and angiogenesis. Based on previous reports, we hypothesized ACGs would down-regulate the Notch signaling-pathway, and thus inhibit these behaviors.

In the current study, we demonstrated for the first time that treatment with ACGs decreased Notch1 expression in a dose-dependent manner in OVCAR5, and higher concentrations of ACGs similarly decreased the expression of the same receptors in OVCAR8. It was additionally found that treatment with ACGs decreased proliferation in these same cell lines.

Cancer metastasis is dependent on the cells' migratory ability and proliferative capabilities. And it was found by wound-healing assay demonstrated that ACGs inhibit the migratory activity of ovarian cancer cells *in vitro*. Together with the noted anti-proliferative effects of ACGs, this suggests they may be effective at preventing metastasis. These effects may be explained by the reductions observed in p-Erk1/2 and Notch1 following treatment with ACGs, both involved in pathways that modulate cancer cell migration and proliferation.

The results presented here indicate that ACGs isolated from *Annona muricata* act as anti-metastatic agents that can attenuate the proliferation of HGSOc, and they may provide a novel therapeutic approach against HGSOc. With few treatment options available, ACGs have the potential to greatly improve patient outcomes, although further studies will be necessary to confirm their effects *in vivo*.

## Translational significance

ACGs isolated from plants of the genus *Annonaceae* have been shown to have antitumor effects in cancer cell lines. However, the molecular signaling pathways underlying this effect are not fully understood. Our findings suggest a regulatory effect of ACGs derived from soursop fruit pulp on Notch signaling pathway in HGSOc, suggesting they may pose a potential therapeutic approach against ovarian cancer.

## References

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