Does angiopoietin-like protein 4 (ANGPTL4) plays a role in apoptosis?

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NANYANG TECHNOLOGICAL UNIVERSITY

URECA *Undergraduate Research Experience on CAmpus*

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Introduction

Anoikis is apoptosis induced by the loss of cell adhesion, which is involved in tissue homeostatsis, wound repair and cancer metastasis. It has been shown that the expression of Fasting Induced Adipose Factor (FIAF), also known as angiopoietin-like protein 4 (Angptl 4), is up-regulated by PPARδ in epidermis during the process of wound healing. This suggests a possible role of FIAF in keratinocytes proliferation, migration and anoikis (apoptosis). However, the precise role of FIAF in anoikis remains to be examined.

Aim

- To investigate the role of FIAF in Anoikis
- To examine the effects of anti-FIAF on HaCaT cells.
- To examine the effects of recombinant FIAF protein on FIAF-knockdown cells.

Method

a) HaCaT (Immortalised human keratinocytes)
b) FIAF-knockdown HaCaT

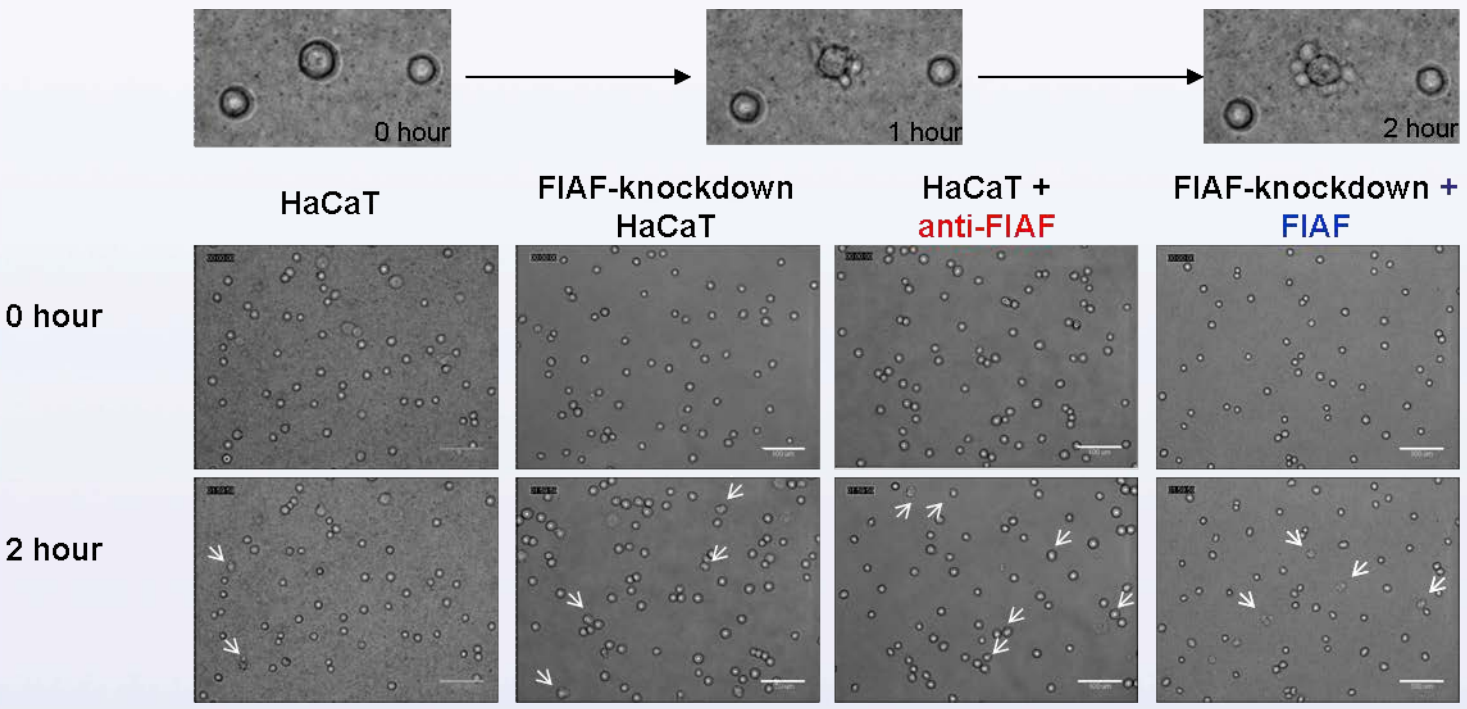
→ Seed cells on agarose-plated petri dish (prevent cell adhesion)

→ (1) Life cell imaging microscopy → Metamorph for analysis

→ ApoAlert annexin V-FITC Apoptosis Kit → (2) Fluorescence microscopy

Results

(1) Life cell imaging microscopy



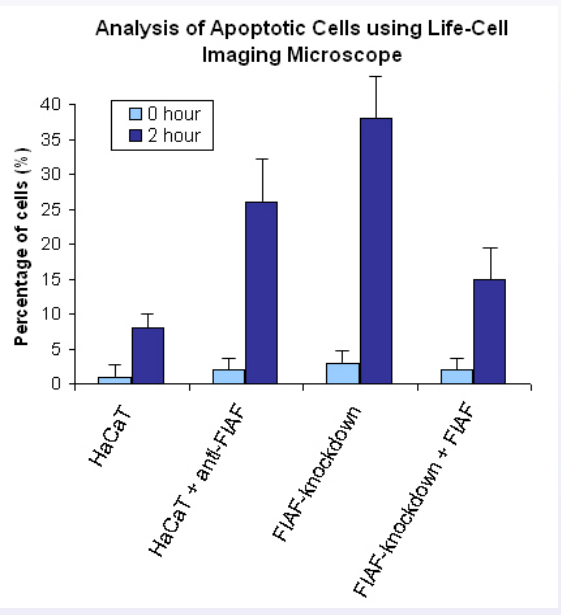
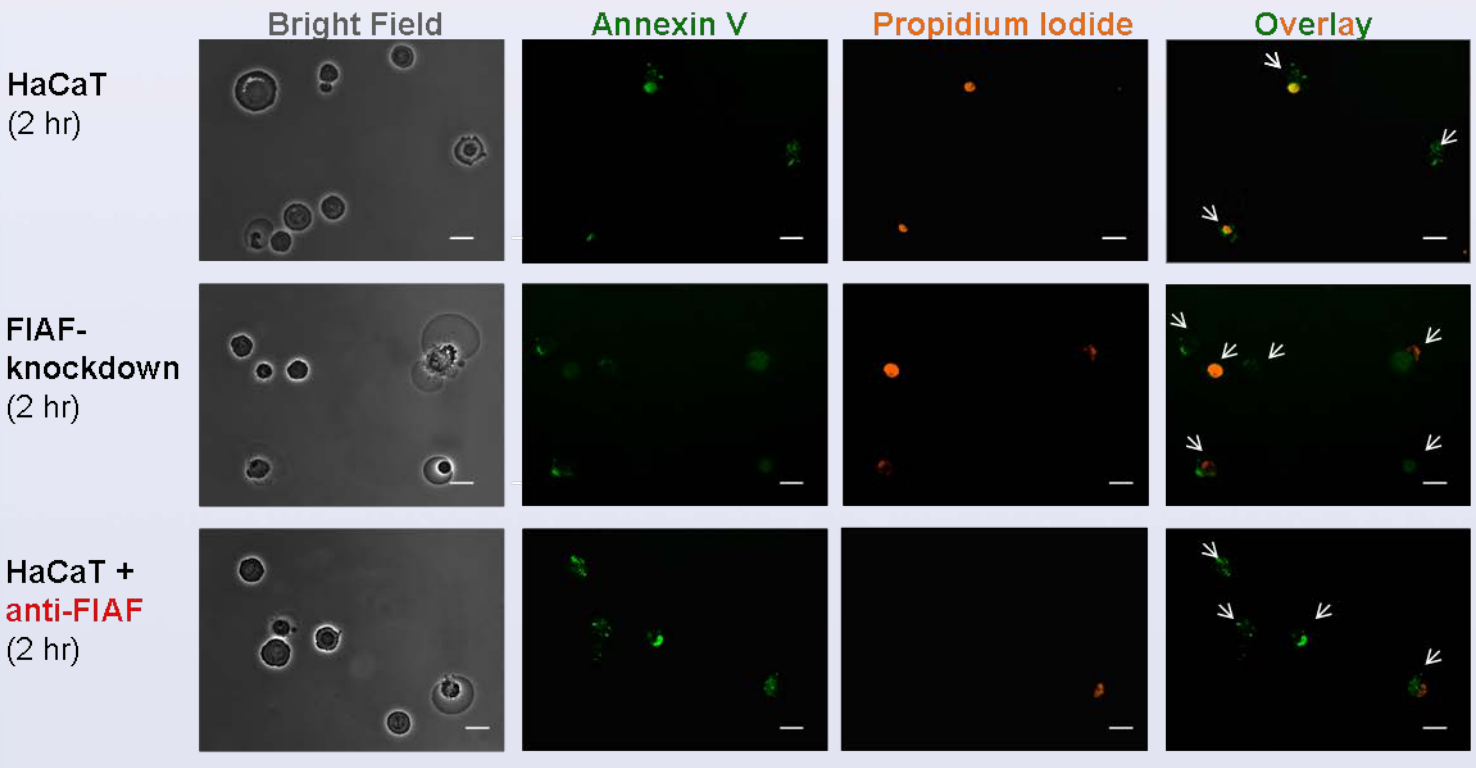


Figure 1. Analysis of Apoptotic Cells. *Detection of apoptotic cells (bleb) using life cell imaging microscopy*

(2) Fluorescence microscopy



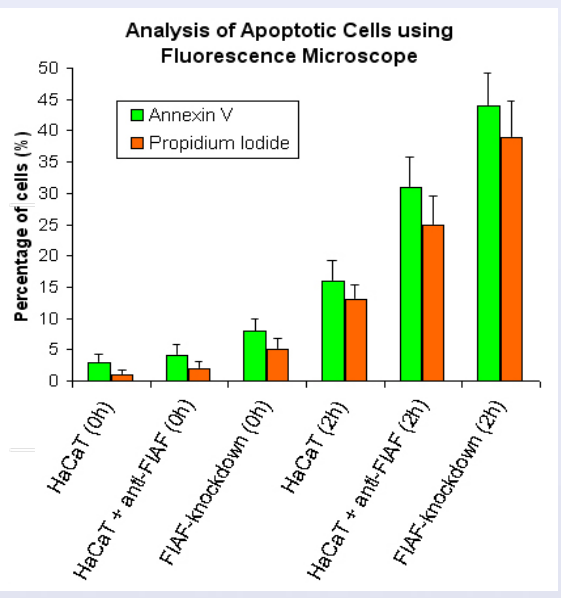


Figure 2. Analysis of Apoptotic Cells. *Detection of early and late apoptosis using Annexin V and Propidium Iodide respectively*.

Discussion

It is observed that FIAF-knockdown cells show a significant increase in the number of apoptotic cells after 2 h of anoikis as compared to control cells. Treating FIAF-knockdown cells with recombinant FIAF protein (2μg/ml) greatly reduce the number of cells undergoing apoptosis. Consistent with the above findings, treating control HaCaT cells with anti-FIAF antibodies (2μg/ml) has the opposite effects. This indicates that the deficiency in FIAF results in increase cellular susceptibility to anoikis, and that FIAF confers an pronounced anti-apoptotic role in epithelia cells. It is noteworthy resistance to anoikis contributes to tumor cells metastatic efficiency while the tumor cells are bloodborne. Thus, FIAF may be a potential alternative anti-tumor target. Future works will investigate on its mechanism of action.

School of Biological Sciences
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