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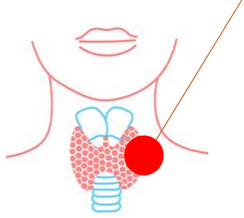
Investigation of the mechanism of anoikis resistance involving protein X in thyroid carcinoma cells

Phichamon Phetchahwang[1,*], Kittirat Saharat[1], Kriengsak Lirdprapamongkol[1], Jisnuson Svasti[1,2], and N. Monique Paricharttanakul[1]

[1] Laboratory of Biochemistry, Chulabhorn Research Institute, Bangkok 10210, Thailand
[2] Applied Biological Sciences Program, Chulabhorn Graduate Institute, Bangkok 10210, Thailand
*Email: phichamon@cri.or.th

Introduction



Thyroid cancer is the most prevalent endocrine malignancy and also the most common head and neck cancer. Mainly, thyroid cancer is classified into 4 types, namely follicular (FTC), papillary (PTC), medullary (MTC) and anaplastic (ATC) carcinoma [1]. Thyroid cancer accounts for 96% of all endocrine malignancies and is particularly more common in females (1-6%) than in males (1-2%) [2,3].

Anoikis is the induction of apoptosis in cells upon the loss of attachment to the extracellular matrix (ECM) and neighboring cells (Figure 1). Cancer cells must acquire anoikis resistance during metastasis [4,5, and 6].

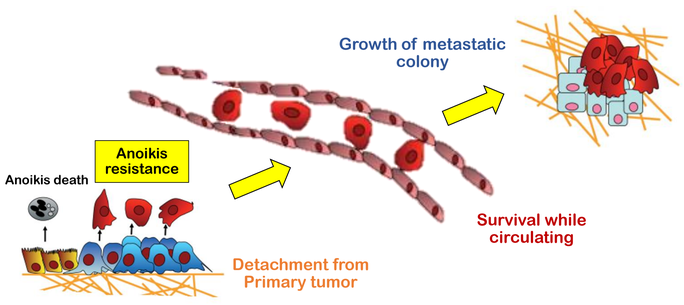


Figure 1 Anoikis resistance is a critical step in cancer metastasis
Modified from: Adeshakin, F. O., et al. (2021).

The survival rate of thyroid cancer patients directly correlates with the development of metastatic stage. Impairing anoikis resistance is widely accepted as a promising strategy for metastatic cancer therapy. Previously, we have discovered an important role of protein X in anoikis resistance of thyroid cancer cells. However, the mechanism of how anoikis resistance is regulated by protein X is unknown.

Aims

To investigate the underlying molecular mechanism of anoikis resistance regulated by protein X in thyroid cancer cells.

Methods & Results

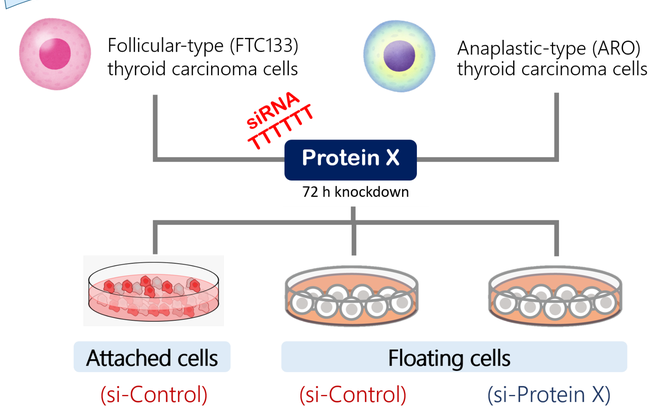


Figure 2 Cell culture & siRNA knockdown of protein X

We studied the role of protein X in anoikis resistance of follicular-type (FTC133) and anaplastic-type (ARO) thyroid cancer cell lines by using siRNA silencing technique. Cells were harvested and subjected to further analyses after siRNA transfection for 72 hours (Figure 2).

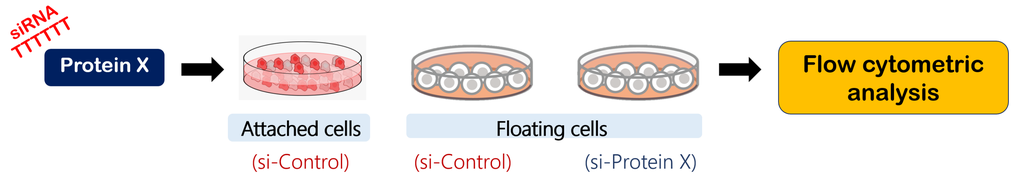


Figure 3 Apoptosis/ anoikis detection by flow cytometry using Muse annexin V & dead cell kit

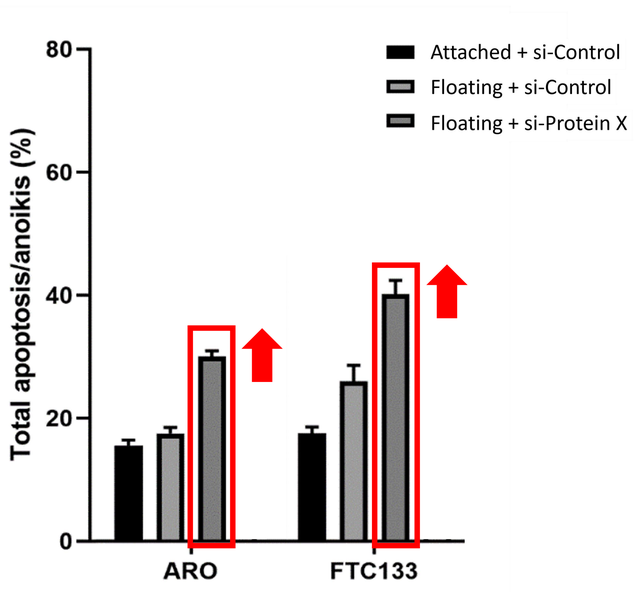


Figure 4 Percentage of apoptosis/anoikis in ARO and FTC133 thyroid cancer cell lines after siRNA transfection.

Effect of protein X silencing on anoikis resistance was determined by using flow cytometric technique (Figure 3). The results showed that the percentage of dead floating cells or anoikis was increased in both cell lines after protein X silencing compared to si-Control transfected cells (Figure 4).

Methods & Results

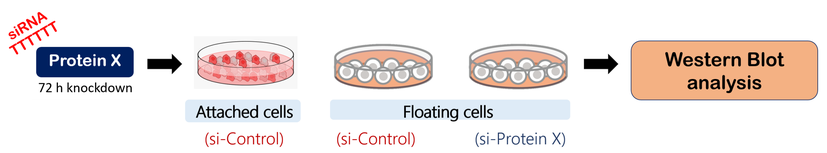


Figure 5 Detection of death receptors A and B protein expression by western blot analysis

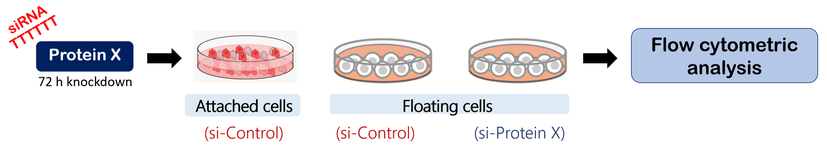


Figure 6 Analysis of cell surface expression of death receptors A and B in cancer cells by flow cytometry

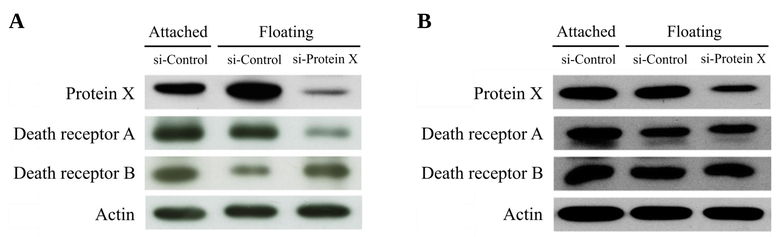


Figure 7 Effect of protein X silencing on the protein expression of receptor A and receptor B (A) ARO and (B) FTC133 cells. Actin was used as a loading control.

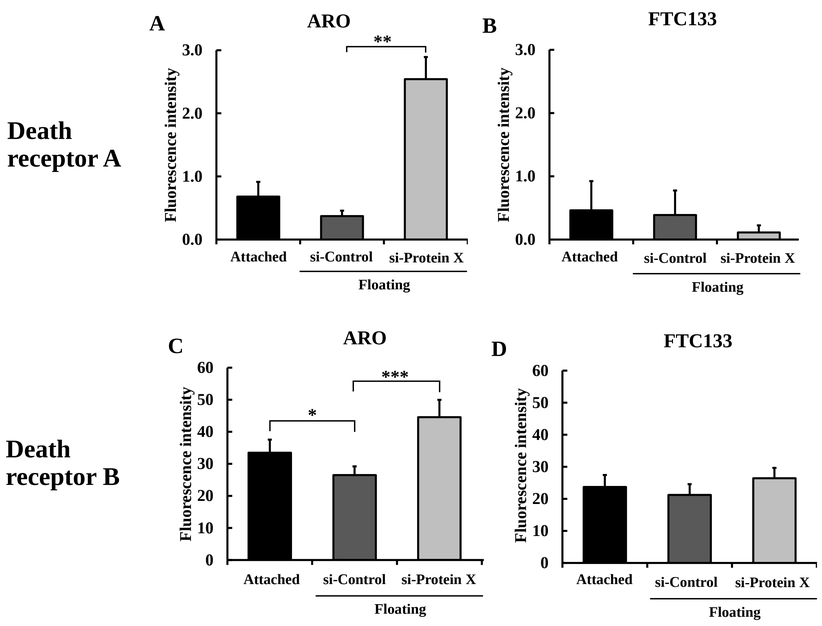


Figure 8 Effect of protein X silencing on cell surface expression levels of death receptor A (A,B) and death receptor B (C,D) in ARO and FTC133 cells. *p<0.05, **p<0.01, ***p<0.001

Death receptors are cell surface proteins that contribute to the extrinsic pathway of apoptosis [7]. Death receptors A and B have been reported as key determinants of anoikis induction in cancer cells [8].

Therefore, we determined the changes in the expression levels of death receptors A and B in protein X-silenced thyroid cancer cells (Figure 5 and 6).

The results showed that The expression of protein X was decreased in both cell lines after protein X silencing compared to that in si-Control transfected cells.

The expression of death receptor A was decreased in ARO floating cells, while the expression of death receptor B was increased, compared to that in si-Control transfected floating cells (Figure 7A).

In FTC133 cells, the expression of death receptors A and B did not change after protein X silencing, compared to that of si-Control transfected floating cells (Figure 7B).

In ARO floating cells, protein X silencing elevated cell surface expression of death receptors A and B, compared to si-Control transfected floating cells (Figure 8).

These results demonstrated that there is a correlation between the elevated cell surface expression of death receptor B and the increased anoikis rate after protein X silencing in ARO floating cells.

Conclusions:

Protein X might suppress anoikis in anaplastic-type thyroid cancer cells by down-regulating cell surface expression of death receptor A and B, consequently promoting thyroid cancer metastasis (Figure 9). Further studies to identify protein X inhibitor(s) will help to treat thyroid cancer metastasis, therefore reducing the mortality of thyroid cancer patients.

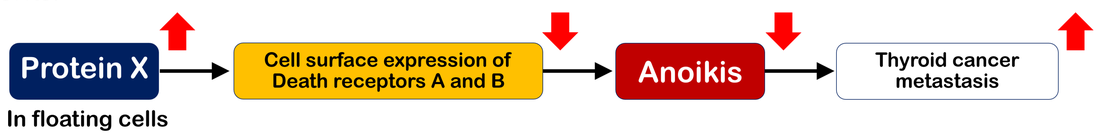


Figure. 9. A proposed mechanism of protein X-mediated anoikis resistance in anaplastic-type thyroid cancer cells.

Acknowledgements:

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References:

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