The Effects of TNF-α and EGF on Epithelial-Mesenchymal Transition in Endometriotic Epithelial Cells

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Background

Endometriosis is a female reproductive disease characterized by inflammatory lesions in the peritoneum derived from infiltrating uterine cells. Epithelial-mesenchymal transition (EMT) is an important process in the formation of endometriotic lesions and involves both endometrial and peritoneal cells. A key to understanding EMT is through determining how protein and gene expression change throughout this shift from epithelial to mesenchymal cells.

Objectives

- Compare how EGF and TNF-α induce and promote EMT in 12Z endometriotic cells
- Assess the effects of TNF-α and EGF on the expression of cytokeratin 19, an epithelial marker
- Analyze changes in gene expression in 12Z cells throughout the process of EMT

Materials and Methods

The 12Z epithelial endometriotic cell line was grown to 100% confluency in 6-well plates and a scratch wound was made through the monolayer of cells. The cells were then treated with EGF or TNF-α and photos were taken at 0, 6, 10, 18, and 24 hours. Protein and RNA were collected at 0, 6, and 24 hours from all treatment groups (n=4). A western blot was run in order to view the expression of cytokeratin 19 among the different time points and the membrane was also blotted with GAPDH to determine equal protein loading.

Results

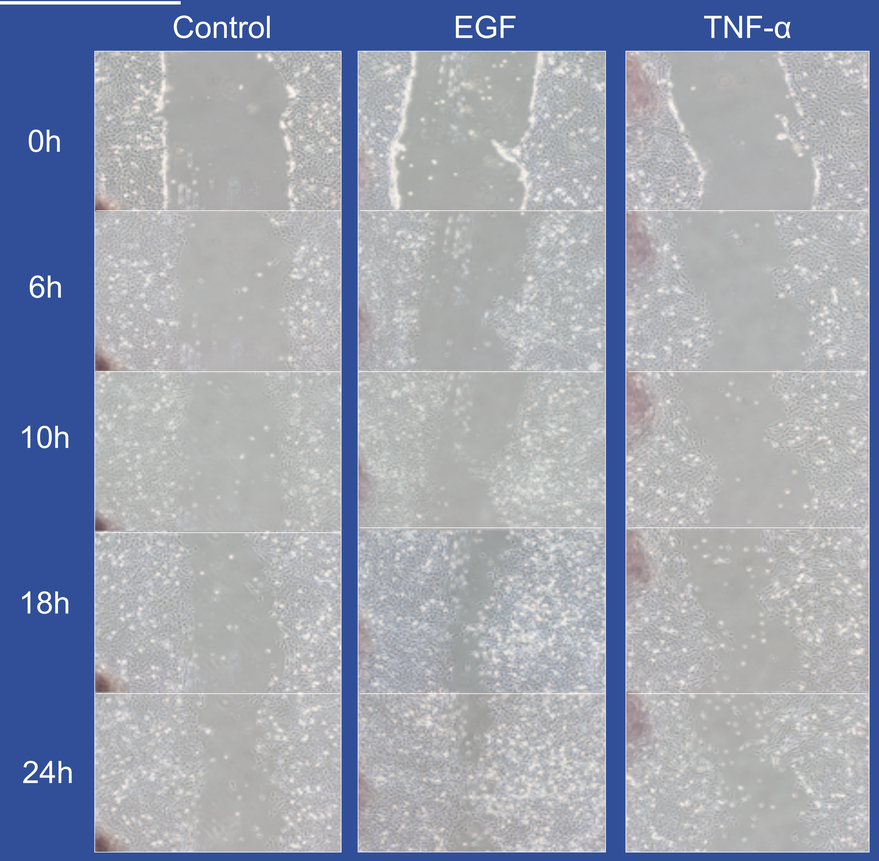

Figure 1. Monolayers of endometriotic epithelial 12Z cells were grown to 100% confluency in 6-well plates and a scratch was made through each with a sterile pipette tip. Cells were then treated with EGF (10 ng/mL), TNF-α (15 ng/mL), or treated with 0.1% BSA in PBS (15 ng/mL) as a control. Photos were then taken to observe how quickly the cells migrated to cover the scratch.

Figure 2. Western blot results of treatment of 12Z cells with EGF and TNF-α over a 24 hour period (2A) indicate that, compared with EGF, TNF-α more severely down regulates the expression of cytokeratin 19. The loading control GAPDH (2B) shows equal protein loading.

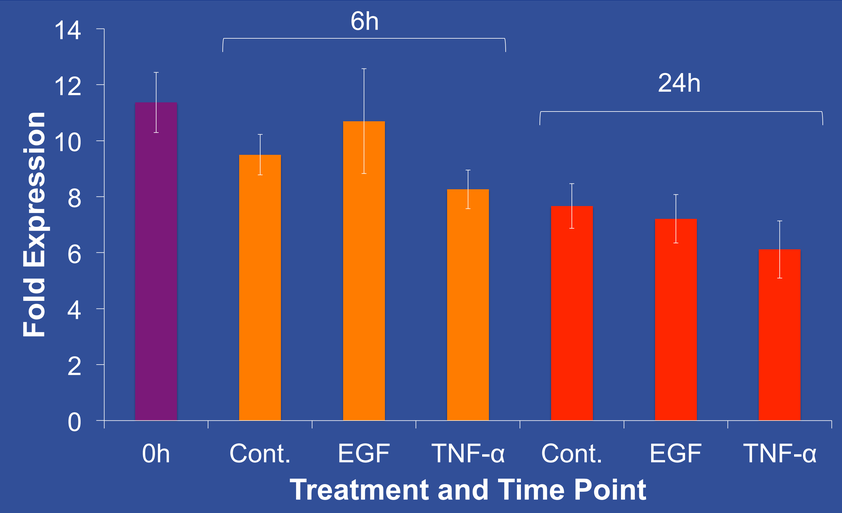

Figure 3. Quantification of the bands from the blot of cytokeratin 19 normalised to the blot of GAPDH shows that there is a steady decrease in the expression of cytokeratin 19 over time and the lowest expression is at 24 hours after treatment with TNF-α.

Conclusions

Cells treated with TNF-α or EGF migrated into the scratch wound area faster than untreated, control cells. Cells treated with TNF-α also showed a lower expression of cytokeratin-19 at 24 hours after wounding than EGF and control. Based on these results we conclude that:

- TNF-α and EGF induce and enhance EMT
- Compared with EGF, TNF-α causes a more significant decrease in the expression of cytokeratin 19

Future Work

This experiment scheme will be used to examine several other EMT markers such as vimentin and N-cadherin and how they are affected throughout EMT in these 12Z endometriotic epithelial cells. RNA samples were collected along with the protein samples and the RNA will be used in a microarray to quantify differences in gene expression in response to treatment with EGF or TNF-α.

Acknowledgements

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