Hypoxia Inducible Factor-Alpha Promotes Expression of Pro-tumorigenic Semaphorin7A in 4T07 Mammary Tumor Cells

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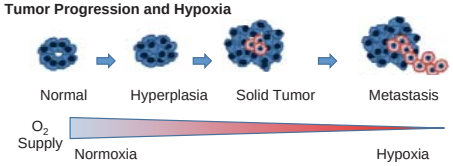
ABSTRACT

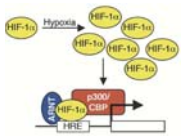
It is estimated that one in eight women will be diagnosed with breast cancer. Developing an understanding of the tumor microenvironment is critical for developing treatments for breast cancer patients. It has been well established that hypoxia, or a lack of oxygen, is fundamental in creating a microenvironment that enables metastasis, via eliciting angiogenic processes. Poorly differentiated blood vessels can fashion an oxygen-deprived microenvironment that triggers the expression of the transcription factor Hypoxia Inducible Factor alpha (HIF-1α) that in turn can up-regulate genes mediating a pro-tumorigenic effect. Our laboratory has discovered that mammary tumors express Semaphorin7A (SEMA7A), a HIF-1α inducible protein. **The objective of this study is to block induction of SEMA7A by HIF-1α in 4T07 mammary tumor cells.** Cobalt Chloride ($CoCl_2$) was used to mimic hypoxia in 4T07 mammary tumor cell cultures. HIF-1α activity in mammary tumor cells was evaluated by use of Hypoxisense 680 fluorescent probe. HIF-1α expression was measured by flow cytometry and SEMA7A levels were assayed by qPCR and ELISA. SEMA7A expression in 4T07 mammary tumor cells increased by 50% in a response to hypoxic stimuli. Further, chetomin pre-treatment dramatically decreased SEMA7A expression in both normoxic and hypoxic conditions. Results suggest that 4T07 tumor cells utilize HIF-1α under both normoxic and hypoxic conditions to modulate SEMA7A expression. Determining the role of SEMA7A in the HIF-1α axis could further elucidate novel pathways in breast cancer.

INTRODUCTION

Tumor Progression and Hypoxia



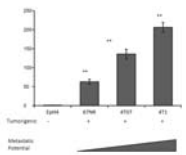
HIF-1α transcriptional complex



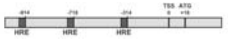
- Hypoxia, or a lack of oxygen, induces activation of transcription factor Hypoxia Inducible Factor alpha (HIF-1α).
- HIF-1α transcribes many genes including those involved in:
  - Angiogenesis -> Tumor Growth
  - Increased cell survival -> Treatment resistance
  - Increased motility and invasion -> Metastasis

SEMA7A in up-regulated in mammary tumors

Increased SEMA7A correlates with malignancy of mammary tumor

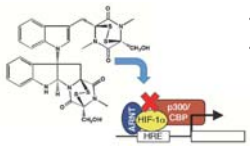


Hypoxia-responsive elements (HRE) in SEMA7A promoter



- SEMA7A is a member of the Semaphorin family an inducible factor that is involved in axon growth signaling, as well as in the immune response
- Our laboratory has shown that mammary tumor express high levels of SEMA7A and inhibition by RNAi retards tumor growth and metastasis.

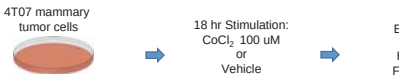
Chetomin inhibits HIF-1α transcriptional complex formation



- Chetomin is a natural metabolite produced by the fungi *Chaetomium*.
- Chetomin interferes with the HIF-1α pathway by preventing binding of HIF1α with transcriptional co-activators p300 and cAMP response element binding (CREB) binding protein (CBP), resulting in attenuating hypoxia inducible expression of transcription.

EXPERIMENTAL STRATEGY

Does hypoxia induce expression of SEMA7A in 4T07 mammary tumors?



Will inhibition of HIF-1α by chetomin decrease SEMA7A expression in mammary tumors?

RESULTS

$CoCl_2$ induced hypoxia increases expression of HIF-1α in 4T07 mammary tumors

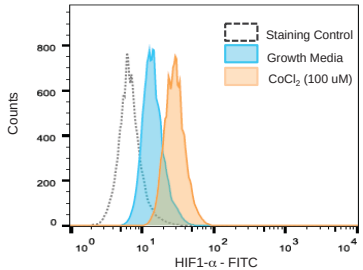


Fig. 1- 4T07 cells were grown to 100% confluency. Cells were fixed, permeabilized, and stained intracellularly with anti-HIF-1α antibody labeled with FITC (RNDsystems Minneapolis, MN). Cells were analyzed in a Calibur Flow cytometer. Flow cytometry results indicate that cells treated with $CoCl_2$ showed an increase in the amount of HIF-1α.

Induction of hypoxia leads to a 50% increase of SEMA7A mRNA in 4T07 mammary tumors

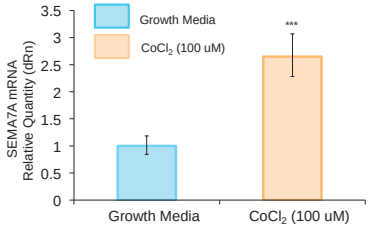


Fig. 2- 4T07 cells were grown to 100% confluency. Results were taken using quantitative real time PCR. PCR showed and increase in the amount of SEMA7A with the 4T1 cells treated with $CoCl_2$ as compared to the untreated cells. Experiments were done in triplicates, $p \leq 0.0001$

Chetomin decreases expression of SEMA7A in 4T07 mammary tumors under normoxic and hypoxic conditions

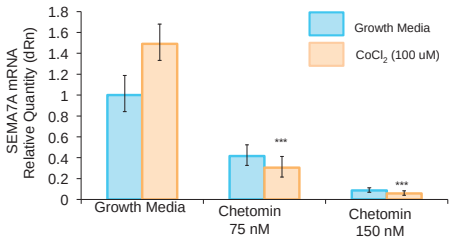


Fig. 3- 4T07 cells were grown to 100% confluency. Cells treated with $CoCl_2$ Cells show a decrease in the amount of SEMA7A. Experiments done in triplicates, $p \leq 0.0001$

Chetomin does not alter the expression of HIF-1α.

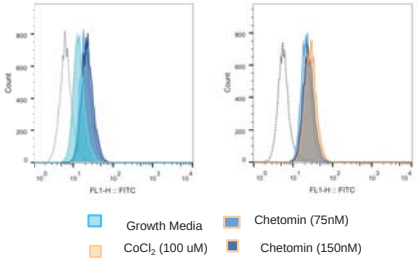


Fig. 4- 4T07 cells were grown to 100% confluency. 4T07 cells cultured with different concentrations of Chetomin and the expression of HIF-1α was analyzed with flow cytometry, using an anti HIF-1α antibody labeled with FITC. HIF-1α expression was not altered under these conditions

CONCLUSIONS

- $CoCl_2$ mimics a hypoxic microenvironment which in turn induces up-regulation of HIF1-α in 4T07 Tumor cells
- Hypoxia induction upregulates the levels of SEMA7A in 4T07 mammary tumor cells.
- Chetomin blocks the activity of HIF-1α in 4T07 cells and decreases expression of SEMA7A in a dose-dependent manner under normoxic and hypoxic conditions
- Chetomin inhibition of SEMA7A is not a result of the degradation of HIF-1α

FUTURE STUDIES

- Correlate expression of hypoxia induced SEMA7A with other pro-tumorigenic/mesenchymal markers
- Use RNAi to inhibit HIF1-α expression to determine impact on SEMA7A expression under normoxic and hypoxic conditions
- Assess the hypoxic microenviorment *in vivo* WT and SEMA7A KO mice
- Determine the efficacy of Chetomin as a SEMA7A inhibitor *in vivo*

ACKNOWLEDGEMENTS

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