GNRHR Signaling in Hematologic Cancer Cell Lines

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Background and Introduction

- Hematologic cancers account for roughly 10% of cancers in the United States.
- Paclitaxel is a microtubule-stabilizing chemotherapeutic drug that has been used against a variety of malignancies including phase I clinical trials for hematological malignancies
- Treatment of acute promyelocytic leukemia cell lines with Paclitaxel (Pax) has demonstrated significant microtubule spindle formation and growth suppression via cell-cycle arrest and apoptotic mechanisms.
- Given the limited information of paclitaxel activity in hematological malignancies, investigations into mechanisms of action and unique signaling pathways remain of critical importance

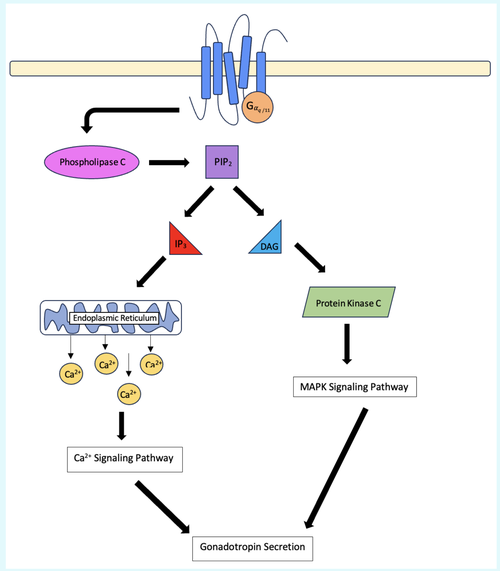

Figure 1. Gonadotropin Releasing Hormone Signaling Cascade

- Gonadotropin releasing hormone (GnRH) plays a role in tumor progression including cell proliferation, angiogenesis, and metastasis.
- GnRH receptor expression has been demonstrated in transformed lymphocytes and extra pituitary tissues, but the effects of GnRH receptor signaling targeting have not been properly investigated.
- Degarelix (Deg) is a GnRH receptor antagonist that is commonly used to treat prostate cancer.

Materials and Methods

- HL-60 Acute Promyelocytic Leukemia cells were cultured in RPMI supplemented with 10% FBS.
- Trypan Blue Exclusion Assay

Rationale and Goals

- GnRHR signaling has been demonstrated to affect cancer cell proliferation.
- The expression of GnRH binding sites in leukemias and lymphomas provides a potential target for chemotherapeutic drugs.
- Analyzing the effect of GnRHR targeted therapy in non-reproductive cancers is a novel and underexplored project that may contribute to the understanding of directed hormonal signaling in hematologic cancers.
- The goal of this project is to elucidate the potential effects of targeting the GnRHR pathway in hematologic cell proliferation.
- Additionally, chemotherapeutic drugs that are not typically used to treat hematological cancers (such as Paclitaxel) may provide useful information about mechanisms of chemoresistance.
- Because of the demonstrated sensitivity of HL-60 cells to Paclitaxel, gradually increasing the dosage from an initially small dose can provide valuable information in regard to the development of chemoresistance to this particular drug.

Results

Figure 2. Initial Viability and Proliferation Analysis. Cells were treated with DMSO (control), Deg (500 ng/ml), Pax (500 ng/ml), or a combination of Deg + Pax (500 ng/ml). Trypan Blue Exclusion Assays were performed to determine cell viability and proliferation at two timepoints. Cells were evaluated for viability and population doublings after 48 hours and 7 days, respectively.

Figure 3. Viability and Proliferation Analysis. Cells were treated with DMSO (control), Deg (500 ng/ml), Pax (500 ng/ml; 100 ng/ml), or a combination of Deg (500 ng/ml) + Pax (500 ng/ml; 100 ng/ml). Cells were evaluated after 48 and 96 hours, respectively. Trypan Blue Exclusion assays were performed to determine cell viability and proliferation.

Results and Discussion

- Treatment with paclitaxel at all timepoints reduced both viability and proliferation with effects increasing as time increased.
- Short-term treatment (48 hour) with Degarelix and Paclitaxel indicated decreased viability compared to either treatment alone.
- The combined effects of treatment on viability and proliferation were less clear at the longer 7-day time points.
- This is likely due to the delayed onset of effect observed for both viability and proliferation following microtubule stabilization.
- In order to better analyze the combinatorial effects, additional doses of Paclitaxel were applied, and analysis was completed prior to 7 days.
- At both the 4-day and 7-day timepoints and regardless of Paclitaxel concentration, treatment with both compounds was less effective than treatment with paclitaxel alone.
- This implies that a reduction in GnRHR signaling could be protective against Paclitaxel treatment but only after Paclitaxel effects build.

Conclusions and Future Directions

- Although the results of this study are inconclusive, the combinatorial effects of Degarelix and Paclitaxel indicate additional investigations are warranted.
- Treatment with Degarelix alone demonstrated the possibility for increased cell viability, and therefore could indicate that the GnRHR signaling pathway plays a protective role in suppressing cancer cell proliferation. Further research concerning targeted therapy of GnRHR signaling is needed to confirm the role of GnRHR in cancer cell behaviors.
- Confirmation of GnRHR effects may have important implications for targeted therapy of resistant hematologic malignancies in patients with low or high levels of GnRHR expression.
- Additional investigations into the interactions between GnRHR and microtubules or microtubule-associated proteins could help to improve our understanding of the interplay between Paclitaxel effects and GnRHR signaling

References

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