Use of G-CSF in Improving Pregnancy Rates in Women with Unexplained Infertility

Anita Washburn
Eastlake High School

Methods and Materials (continued)

A. Study 1: G-CSF influence on immune response and its effect on thin endometrium

G-CSF administration is partly responsible for the promotion of Th-2 cytokine influence crucial gene expression in the endometrium, including local immune cells required for establishing and maintaining a pregnancy.5

Researchers randomly administered G-CSF (1 microg/day) starting on the sixth day after ovulation until the occurrence of menstruation or to the end of the ninth week of gestation. The placebo group (33 women) was treated daily with the same dosage of saline solution for the same duration as those treated with G-CSF.

All the patients conceived without additional intervention within three months from randomization and inclusion in the study. Pregnancy outcome (delivery of a healthy baby without major or minor malformations) was the primary outcome measure.

C. Study 3: IVF (including frequent IVF failure)

Despite major advances in assisted reproductive techniques, the implantation rates still remain relatively low. Some studies have demonstrated that intrauterine infusion of granulocyte colony stimulating factor (G-CSF) improves implantation in infertile women. In this trial, 100 infertile women between 18-40 years old with normal endometrial thickness who were candidates for IVF participated in this study (50 women in each group). In G-CSF group at the day of oocyte retrieval, after oocyte collection, 300 mg of G-CSF was administered by slow transcervical intraterine infusion with IUI catheter. In the control group, the cycles were continued without G-CSF infusion. In all patients, 2-3 embryos were transferred by using an embryo transfer catheter; two days after oocyte retrieval. Pregnancy outcomes, implantation rate, the ongoing pregnancy rate and miscarriage rates were assessed to statistically determine the effect of G-CSF.

Results

A. Study 1: G-CSF influence on immune response and its effect on thin endometrium

The patients with infertility due to a uterine or endometrial factor all showed an increase in endometrial thickness, along with a 25% pregnancy rate increase.

B. Study 2: RM/unexplained infertility

It was found that G-CSF significantly improved the number of live births and miscarriages but did not significantly impact the gestational week of miscarriage and newborn weight.

C. Study 3: IVF (including frequent IVF failure)

In the third trial, G-CSF effect on implantation and pregnancy rates in normal infertile women who were eligible for IVF treatment were evaluated. Researchers found that pregnancy outcomes did not significantly improve after intrauterine G-CSF infusion in women with normal endometrial proliferation. However, researchers observed that the use of G-CSF shows a positive correlation between uterine environment and embryo development in IVF patients.

Discussion

With this groundbreaking preliminary research, it is now important to study G-CSF’s implications and limitations in other fields of reproductive biology and Oncofertility. This study focused on new ways to target and diminish the high percentage of miscarriages and overall infertility. Instead of looking purely at the physiology and possible pathologies that may be causing RM and ovarian failure, this approach combats the issue at onset by preventing an uncontrollable immune response to a “foreign substance” along with strengthening the overall environment through G-CSF's ability to produce stem cells and leukocytes. In testing its effect on implantation and pregnancy rates in IVF, researchers found success in improving the quality and strength of the endometrium. Additional studies are certainly necessary to find how it can improve the success rate in full term pregnancies. Further research with G-CSF can also contribute to better outcomes in treatment of other incurable diseases and improving researchers and clinicians today. Autoimmune disorders such as Crohn’s disease and MS have similar characteristics to those seen in women experiencing unexplained miscarriages, as they target important and natural aspects of the body in a destructive manner.

Acknowledgements

It truly takes a village to create a poster, and I was so fortunate to be apart of and to learn from such inspiring individuals. I can’t thank Dr. Ericka Sengeth-Mitchell enough for all that she has done for everyone of us. She truly is a guiding light for me in both the field of science and life as a whole. I am extremely grateful for the time and effort put into each and every one of the presenters throughout the academy. I especially like to thank Dr. Irene Su and Dr. Jeffery Chang for expanding my love and curiosity for science. To all my ROSA sisters and big sister in science, Yasmim, for their continued support. Finally, I’d like to thank Ms. Patricia Winter and the Oncofertility Consortium for making this program possible.

References


Evaluating the Potential Efficacy of Lipid-Based Polymers as Method of Genetic-Based Therapy for Endometriosis

Ayasha Aslam-Mir
Del Norte High School

Abstract

Endometriosis, a disease in which endometrial tissue grows outside of the uterus, affects 10% of women of reproductive age, causing damage to pelvic organs, intense pelvic pain, and infertility. Current treatments include hormonal suppression and surgical procedures; however, these therapies show promise as a method of less invasive and less obstructive treatment. Lipid and lipidoid conjugates have shown efficiency both in transport of genetic therapies and targeting of endometrial lesions respectively. A polymer micelle system using nanoparticle complexes formed from lipid graft copolymer and a lipid microsphere derived factor (PFD) plasmid were combined as a method of genetic therapy. In a study by Zhu, Hugh S. "MiR214 Overexpression of PEDF or 3 mg of the vector, were injected into the mice. A negative control group was given a 1.5 ml injection of sterile saline IV fluid. A positive control group was orally administered 200 mg of danazol, a suppressor of estrogen synthesis, each day for 2 weeks. The experimental group had 5mg/kg body weight of PFD injected as well and revealed significant growth in apoptosis of endometrial tissues; TGF -β activated protein kinase (MAPK) signaling pathways. This phenomenon suggests that genetic modification of endometrial cells, with the utilization of genetic therapies, could be a promising method for reducing endometriosis.

Results

1. The efficiency of delivery methods for genetic materials can lead to effective targeted therapy and potential cure.
2. The use of lipid-based nanoparticles and micelles as carriers of genetic therapies for endometriosis provides the advantage of enhancing the gene expression in endometrial tissue.
3. The use of lipid-based nanoparticles and micelles as carriers of genetic therapies for endometriosis provides the advantage of enhancing the gene expression in endometrial tissue.

Discussion

The objective of this research is to determine the efficacy of lipid-based polymers in reducing endometriosis progression or growth of genetic expression in endometriosis.

Table 1: Genes Observed in Endometrial Tissue and Corresponding Effects of Altered Expression

<table>
<thead>
<tr>
<th>Gene</th>
<th>Role Based on Observation</th>
<th>Cell Type</th>
<th>Expression</th>
<th>Inhibition Regulation</th>
<th>Effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>TGF-β</td>
<td>Human</td>
<td>Overexpression</td>
<td>Migration, invasion, inflammation, apoptosis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGFA</td>
<td>Wistar Rat</td>
<td>Downregulation</td>
<td>Shrink endometrial implants in test subjects, reduction of microvessels in lesions</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

References

As survival rates of childhood cancers, like leukemia, increase due to more aggressive and new treatments, more children are left with an increased risk of infertility. Options for female cancer patients include oocyte or embryo cryopreservation and ovarian tissue preservation, however the inactivity of the hypothalamic-pituitary-ovarian axis prevents oocyte and embryo cryopreservation in prepubertal leukemia patients. These methods also hold a high risk for reintroduction of malignant cells upon retransplantation. The use of bioengineered ovaries would allow normal function of the ovaries without the risk of reintroducing malignancy. Development of an effective decellularization process to produce an extracellular matrix (ECM) based scaffold is key to the successful implementation of this alternative. In a study testing the effects of Sodium Lauryl Ester Sulfate (SLES) as a decellularizing detergent, ovarian tissue samples were harvested from 18-35 year old patients. The ovarian samples were then bisected and cut into strips of about 2.0 mm. The samples were then decellularized with 1% SLES for 48 hours at 18-20°C. They were then rinsed several times with a phosphate-buffered saline (PBS) to remove remaining chemicals and cells. Hematoxylin and Eosin (H&E) and Hoechst were used to stain the samples to ensure effective decellularization. The ECM was also examined using Heidenhain’s AZAN stain. The cytotoxicity of the SLES was analyzed using cultured human Wharton’s jelly mesenchymal stem cells, to confirm human compatibility. To test the in vivo success of the scaffolds, primary ovarian cells were harvested from 8 week female rats and cultured on the scaffold. After one day, stroma cells, primordial and primary follicles, and oocyte complexes were found. With the confirmation of the effectiveness of the SLES detergent with the decellularization of ovarian samples, there are future possibilities of utilizing bioengineered ovaries to restore fertility in female, pre-pubescent, leukemia patients.

Leukemia is a malignancy that affects the blood and bone marrow of the patient. Leukemia is the most common cancer in children, however survival rates have increased over the years due to more specialized and intense treatments. The increasing survival rates has now turned some of the attention onto fertility preservation of these patients. Currently, techniques such as oocyte and embryo cryopreservation, as well as ovarian tissue preservation, can be offered to female patients to help counter infertility. For leukemia patients who are prepubescent, the options are more limited. Given the nature of leukemia as a blood cancer, there are no available fertility techniques that do not risk the reintroduction of malignant cells. In a study with 26 patients who cryopreserved their ovarian tissues prior to cancer treatment, 75% of the eight patients with chromosomal abnormalities in their malignant cells, there was evidence of leukemia cells in their ovarian tissue. As the risk of reintroducing malignant cells upon transplantation of ovarian tissue in leukemia patients is too high, one promising method relies on the use of Sodium Lauryl Ester Sulfate as a detergent for ovarian tissue, eventually producing a bioengineered ovary.
Effect of hMG on Oocyte Development During In Vitro Maturation

Cassidy Kirk • Madison High School

Abstract
In Vitro Maturation (IVM) is a fertilization procedure in which the prospective mother has her immature oocytes harvested so that they can be fully developed and artificially fertilized in a laboratory. IVM is helpful for women with resistant ovary syndrome because it allows oocyte development even when the patient’s hormones aren’t balanced. Although IVM does improve her ability to have children greatly, having the patient produce enough quality oocytes is a great setback. One approach to this is to stimulate the ovaries by using human menopausal gonadotropin (hMG) since it encourages many follicles to develop but isn’t one of the hormones that aren’t processed properly by the patients. This study compares the quality, measured in fertilization and live birth rates, as well as the quantity, measured in the number of oocytes retrieved, of immature oocytes produced by women with and without hMG. This study resulted in an average of 2.14 cumulus-oocyte complexes (COC) being retrieved without any stimulation compared with 6.43 COC being retrieved using hMG stimulation. This resulted in 54.1% of unstimulated COC being fertilized, and 14.3% used resulting in a live birth, while 54.6% of stimulated COC were fertilized and 16.7% used resulted in a live birth. These results show that although hMG does greatly increase the number of COC retrieved, it has no substantial effect on the quality of oocytes produced. A future step to take would be to investigate which hormones or oocyte media result in the best quality oocytes to maximize the potential of the eggs.

Objective
In this study, we hope to reveal what factors hMG manipulates when used as an ovulatory stimulant during IVM. This element of IVM is particularly pivotal, because when we identify the areas it improves, we can further research the hormones, procedures, or environmental factors to give patients the best ability to protect the health of the mother and her child.

Methods and Materials
In the “Endocrine” study, all patients with normal functional ovarian reserves (NFOR) were given a standard mixture of 8.1 ml/mL of FSH and hMG. While in this study, the data was used to compare the fertility and treatment of women with and without NFOR, the data of NFOR remains useful while comparing to other studies with standard treatment, but no ovarian stimulation used. The participant group of this study contained 10 NFOR patients. The “Elsevier” study followed 1,187 live birth pregnancies and chose to administer each fertility treatment to women with NFOR. It was measured that the mean gestational age at delivery for women without NFOR, hMG and hCG Stimulated the ovaries, and used no other ovulatory stimulants. The administration of the hMG begins on the 8th day of the patient’s cycle. This study used the same treatment during each pregnancy, but measured the gestational age at delivery, birth weight, and Apgar score compared with the number of children per pregnancy (ie. Singleton, twins, triplets, or quadruplets). The “Journal of Assisted Reproduction and Genetics” measured the oocyte retrieval rate, success of oocytes, and embryo success of non ovulation-stimulating cases and cases using hMG to stimulate the patients’ ovaries. This study used 150-300 IU/day for 7 days, beginning on the 2nd day of the patient’s cycle. As in the “Elsevier” study, the variation of hMG administered was based on the oocyte development level seen. This study contained 16 patients and also looked at the results of using hMG as an ovulatory stimulant for patients with deficient ovary maturation.

Figure 1: HCG Unstimulated COC Results Adapted from Galvão et al, 2018

![Figure 1: HCG Unstimulated COC Results Adapted from Galvão et al, 2018](image1.png)

Figure 2: HCG Stimulated COC Results Adapted from Galvão et al, 2018

![Figure 2: HCG Stimulated COC Results Adapted from Galvão et al, 2018](image2.png)

Results
The study from the “Journal of Assisted Reproduction and Genetics” showed that patients who received hMG ovarian stimulation produced an average of 6.43 COC per patient and patients with no ovarian stimulation produced 2.14 COC each. These COC led to the fertilization 54.6% of stimulated COC being successfully fertilized compared to 54.1% in unstimulated patients. Finally, the 16.7% of stimulated COC resulting in a live birth versus 14.3% of unstimulated COC.

In the “Elsevier” study, it was measured that the mean gestational age at delivery for singletons was 37 weeks and 4 days across 960 pregnancies. It also showed that the interquartile of babies scored a 7-9 on the Apgar test at 1 minute after birth and the interquartile scoring a 9-10 5 minutes after birth. It shows that although these babies who were born from stimulated ovaries don’t handle to birthing process perfectly, they do adjust quickly and well to live outside of the uterus.

Discussion
The “Journal of Assisted Reproduction and Genetics” study has helped in the effort to make ART more efficient and safer. One aspect it influences is the stimulation of the ovaries, and how they are receptive to hMG, but no other major reproductive hormones. This could be the result of a structural difference or the role it performs, that happens to coincide with high follicular development. This hormone, and others like it are gateways for the rejuvenation of resistant ovary syndrome patients. The “Elsevier” study provides much needed insight into the effect that hMG has on the development during gestation and health of the bay after birth, and that there is no real negative effect on the growth and health of the child.

Implications
The results of this study are welcoming others of its kind, looking into hormones and treatments that could interact with the reproductive system in a similar way to hMG, in hopes of finding something that gives this patient population an even better chance of producing large yet healthy quantities of eggs. Because it was shown that hMG did not lead to any improvement in the quality of eggs as well as the live birth rate, success of oocytes, and embryo success of non ovulation-stimulating cases and cases using hMG, this study could also inspire further research into other stimulants or oocyte media that promote healthy egg and embryo growth. Finally, these results encourage IVM providers to adopt the use of hMG as a stimulant, and possibly even for a wider patient population group, such as including women with oocyte development disease such as polycystic ovary syndrome.

Acknowledgements
Bundles of thanks to all the family, friends, and teachers, who supported me while making this poster and in the past.

References
Capturing Excess Doxorubicin with 3D Printed Absorbers to Minimize Side Effects of Chemotherapy

Claire Wang • Torrey Pines High School

Background and Objective

Cancer is becoming the leading cause of death in most developed countries. Doxorubicin is a common chemotherapy drug and is given by injection into a vein. Although it is known that there is a positive linear correlation between the dose of doxorubicin and the number of tumor cells death, the dosing often limited due to the systemic toxic side effects. Common side effects include hair loss, bone marrow suppression, and vomiting. More serious side effects may involve tissue damage at the site of injection and dilated myopathy, which results in congestive heart failure. A novel approach to mitigating these side effects is inserting 3D printed absorbers into the draining veins of the organ that the tumor is located in. The absorbers capture doxorubicin after it has had an effect on the target tumor.1

Abstract

Cancer is a major health problem worldwide and is the second leading cause of death in the United States.4 However, doctors are forced to limit the dosage of drugs in chemotherapy, particularly doxorubicin, due to its toxic side effects such as skin eruptions, diluted cardiology and heart failure.2 During intra-arterial chemotherapy infusion to a target organ, excess drugs that do not remain in the target organ pass through and circulate to the rest of the body.1 Typically, over 50%-80% of injected drugs pass by the tumor and enter general circulation.6

One approach to mitigating this off-target damage is to insert a 3D printed absorber into the draining veins of the organ that contains the chemotherapy-targeted tumor through a microsurgery. The absorber absorbs excess drugs before it enters the systemic circulation. The device contains a hole through the length of the cylinder that allows the insertion of the device with minimal invasive image-guided endovascular surgical procedures. The porous cylinder structure was printed by the cross-linking of PEGDA. Inside the structure is a square lattice structure that is coated in polystyrene sulfonate, which binds to the widely used chemotherapy drug doxorubicin that also induces significant side effects. The introduction of the absorbers into the blood of swine models undergoing infusion in the common iliac vein of 50 mg of doxorubicin over 10 minutes enabled the capture of 64 ± 6% of the doxorubicin without any immediate any noticeable effects. Doxorubicin concentrations in blood samples were determined using fluorescence spectroscopy. Moving forward, further decreasing lattice size and changing the chemical composition and thickness of the coating layer may enhance drug capture. In future human trials, absorbers can be customized to fit optimally in the veins of the patient by doing a pre-procedure MRI.4

Methods and Materials

Tiny, porous cylinders were 3D printed with the internal structure shown in Figure 2. The absorbers were 5 mm in diameter and 30 mm in length to depth chartre proof of concept. The diameter was determined by the 6 mm diameter of the introducer sheath that is used in surgery. The cylinder has a hole that runs through the entire length of it; this central hole allows for the attachment to a guide wire that is used for minimally invasive surgery.

Results

The experiment involved two groups: a control group wherein uncaptured doxorubicin was deployed, and an experimental group with the coated absorbers.1

Control group: Uncaptured doxorubicin was placed in the common iliac vein, and sampling catheters were placed in the three locations described above. As shown in Figure 4, the doxorubicin concentrations at the pre-device and post-device locations are similar; therefore, it can be concluded that a majority of the injected doxorubicin passed through the absorbers. At both locations, the doxorubicin concentration increases rapidly at early times and maintains its level for about 10-15 min. Then, the levels decrease to zero at about 30 min. The doxorubicin concentration measured at the peripheral location only showed a slight increase when doxorubicin was injected. Figure 4b shows images of plasma from centrifuged samples obtained from the three catheters during the control experiment. As doxorubicin has a characteristic orange color, a darker orange color indicates a higher doxorubicin concentration.

Experimental group: When the coated absorbers were used, as shown in Figure 4c, post-device doxorubicin concentrations is significantly lower than that measured at the pre-device location. 69% of doxorubicin was captured by the 3D printed absorbers. Pictures taken of the centers for one plasma sample taken from the three catheters confirm the removal of doxorubicin. Additionally, no side effects relating to biocompatibility or blood flow were observed.

The described experiments were repeated in two additional animal models. The results were similar to the previous trials.

References


Acknowledgements

First and foremost, I would like to thank Dr. Ericka Senegar-Mitchell for her endless enthusiasm and infectious passion for science and for being the most inspiring big sister in science. A big thanks to Dr. Jeffrey Chang, Dr. Irene Su, and all of our guest lecturers for imparting their knowledge to us. Ms. Patricia Winter for organizing this amazing program; and Yasmine for always being there and supporting us. Finally, I would like to thank all of my ROSA sisters for an incredible summer and beyond.

Relevance to Biotechnology

Structurally simple polymers can be utilized in making new biomedical devices aimed at increasing the efficiency of chemotherapy. In this study, this device, called the ChemoFilter, was used with treatment for hepatocellular carcinoma.7

Figure 5. Treatment of liver cancer by administering intra-arterial chemotherapy via the hepatic artery.

Implications

Researchers have built and deployed 3D printed absorbers that capture excess doxorubicin in vivo before it enters the systemic circulation and causes side effects. The polystyrene sulfonate coating on the internal lattice structure allows for drug capture. Initial testing has shown the capture of 64 ± 6% of doxorubicin without noticeable side effects in the ICU. The 3D printed device can be enhanced by decreasing the lattice size and thus increasing surface area for drug binding. Changing the chemical composition and thickness of the coating can potentially increase efficacy as well. In future clinical trials, pre-procedure MRI or CT datasets can help the 3D printed absorbers fit even more optimally into the patient’s veins. Although there is still a lot of optimism to be had for the current results are promising; this approach opens a new pathway to help patients fight cancer by minimizing drug toxicity and enabling high-dose chemotherapy.

Figure 1. The proposed approach of 3D printing an absorber for drug capture.4

Figure 2. Design of the 3D printed porous cylinder using computer-aided design (CAD) and optic micrographs of its interior.4

Figure 3. Magnified views of the uncoated (gray) and coated (orange) absorbers at different locations.4

Figure 4. Results of the in vivo experiments: (left) uncoated control absorbers and (right) two coated absorbers. Doxorubicin concentration in the blood as a function of time at the three different sampling locations for (a) uncaptured absorbers and (b) coated absorbers. Photos of plasma from the centrifuged samples of (b) the uncoated absorbers and (d) the coated absorbers.4

Figure 4. Results of the in vivo experiments: (left) uncoated control absorbers and (right) two coated absorbers. Doxorubicin concentration in the blood as a function of time at the three different sampling locations for (a) uncaptured absorbers and (b) coated absorbers. Photos of plasma from the centrifuged samples of (b) the uncoated absorbers and (d) the coated absorbers.4

In vivo experiments were performed with the coated 3D printed absorbers in three swine models. The swine models were undergoing chemo-infusion of 50 mg of doxorubicin in the coronary arteries over 20 minutes. The doxorubicin concentration in the common iliac vein of 50 mg of doxorubicin over 10 minutes enabled the capture of 64 ± 6% of the doxorubicin without any immediate any noticeable effects. Doxorubicin concentrations in blood samples were determined using fluorescence spectroscopy. Moving forward, further decreasing lattice size and changing the chemical composition and thickness of the coating layer may enhance drug capture. In future human trials, absorbers can be customized to fit optimally in the veins of the patient by doing a pre-procedure MRI.4
**Objective**

Currently, ovarian tissue transplantation is the main fertility treatment option for prepubescent female cancer patients undergoing cytotoxic treatments. However, it poses the risk of reintroducing malignant cells, which may reduce the ability to grow primordial follicles in vitro is vital because they are abundantly present in females of all ages, thus allowing for in vitro maturation of oocytes followed by IVE. During primordial follicle growth, a signaling molecule called basic fibroblast growth factor (bFGF) assists ovarian granulosa, stromal and theca cell proliferation and cumulus cell apoptosis inhibition, cumulus expansion, and meiotic resumption. The relevance of this research lies in its potential to improve the survivalivity and maturation rate of human follicles in vitro. This research would not have been possible without advancements in biotechnology, including tissue-engineering of a 3D alginate matrix and advancements in Assistive Reproductive Technology (ART). The implications of these advancements are monumental, reducing the high costs associated with routine in vitro fertilization and improving the quality of life of thousands of patients worldwide suffering from infertility.

**Methods and Materials**

**Experiment 1:** Ovarian tissue from 4 females aged 6–38 years were cut into 1–2mm slices and were frozen, thawed, fixed in Bouin’s solution, and placed in Millicell inserts coated with 3% Alginate and cultured in media consisting of αMEM with 0.47 M ribonucleotides, 2 mM/L streptomycin, 60 ng/mL penicillin and 10 ng/mL epithelial growth factors. Each of the experimental groups received either Retinoic Acid (RA) (2 μM), bFGF (20 ng/mL) or a combination of RA and bFGF. After 24 hours, fixed specimens were assessed for cleavage to the two-cell stage. 1

**Experiment 2:** High doses of basic FGF enhanced follicular development at rates significantly higher than the thawed control (Figure 2). In addition, E2 secretion (levels varied between 205 and 801 pg/mL) increased significantly between the first and second culture week with the addition of basic FGF, indicating the role of FGF in the proliferation of early follicles. 2

**Experiment 3:** Larger diameter and increased survival rates of follicles grown with 200 ng/mL bFGF (Figure 3). 3

**Experiment 4:** After 8 days in culture, all 154 follicles had increased in size. The diameter and survival rate of follicles in the group of 0 ng/mL bFGF was significantly higher than those in the group of 0 ng/mL bFGF (Figure 3). 3

**Discussion**

BFGF, which plays a vital role in embryonic development, cell growth, and morphogenesis, was demonstrated to have similar effects on in vitro maturation of oocytes in culture systems. Utilizing 3D alginate culture systems has been a reliable method of in vitro maturation. However, the success rates of follicles, especially primordial follicles, reaching Metaphase II has been low, and the live birth rate even lower. 4 The addition of bFGF not only increased the rate at which follicles developed, but also significantly improved the survival rate of human follicles. This research lays the groundwork for utilizing the individual or combined effects of multiple growth factors including bFGF on follicle development to improve the culture medium, in addition to developing sequential culture media with various growth factors for the different features of various follicle stages in human follicular development.

**References**

Objectives
The purpose of this poster is to assess and evaluate the effect that cryopreserved ovarian tissue can have on prepubertal women. Studies have shown that prepubertal female cancer patients can use cryopreserved ovarian tissue to induce puberty. The purpose of inducing puberty is to start ovulation, giving young girls the option to preserve their eggs and have higher chances in saving their fertility.

Abstract
There is a group that faces the greatest challenge in protecting their fertility, and that is the pediatric and prepubertal female cancer patients. Girls who have not reached puberty yet are not ovulating, therefore there are no eggs to freeze or preserve. As of now, the only option is to cryopreserve their ovarian tissue. However, there have been recent cases of puberty being induced by the transplantation of cryopreserved ovarian tissue. A case of ovarian tissue auto-transplantation with fertility restoration resulted in a live birth as the tissue was collected at an age of 13 years and 11 months, before ovarian tissue auto-transplantation with fertility restoration resulted in a live fertility.

Methods and Materials
In a case report from the Demeestere lab, a 27 year old woman underwent ovarian tissue transplantation with tissue she cryopreserved at the age of 13, before she had gone through puberty. A right oophorectomy took place by laparoscopy in 2001, and 62 fragments of ovarian tissue were cryopreserved. In 2011, she wished to become pregnant and underwent ovarian tissue transplantation. Up until this point, she had never had a menstrual cycle or period. Using the da Vinci surgical robotic system, four tissue fragments were grafted on her left ovary, six were grafted in the right peritoneal bursa, and five were grafted using a trocar incision. In a case from the Ernst lab, a 9 year old girl diagnosed with Ewing Sarcoma cryopreserved 10 fragments of ovarian tissue from both of her ovaries. At the age of 13, she showed high FSH levels, and no sign of pubertal development. She decided to transplant 2 fragments back into her remaining ovary. And because her gonadotropin levels were high, follicular development was expected to be stimulated. In another case by Catherine Poiriot, a 10 year old girl with sickle cell disease cryopreserved 23 fragments of ovarian tissue by a right oophorectomy. At the age of 13, she came back for the autotransplantation of the tissue in an attempt to induce puberty. An abdominal pocket was made where 3 fragments of tissue were transplanted.

Results
For the first report, the woman’s FSH levels decreased while her estradiol levels increased following the transplantation. Four months later, her hormones reached premenopausal levels, and after five months, she had her first menstrual cycle. Her cycles started to become more consistent, and after two years, she had a spontaneous pregnancy with her partner. For the second report, the girl saw similar results to the case before. Her FSH levels decreased 4 months after the procedure, and her estradiol and inhibit B started to increase to premenopausal levels. One year after the transplantation, the girl had regular menstruation, but 6 months later her FSH increased to postmenopausal levels. However, her ovarian function was confirmed when a few antral follicles were observed to have diameters of 5-6 mm. For the final report, the young girl experienced breast growth immediately, and reached Tanner Stage S2 in 4 months. After 2 years, she had finally reached regular menstrual cycles and irregular thereafter.

Discussion
Transplantation of Cryopreserved Ovarian Tissue is not only paving the way for fertility but also giving women their womanhood back. The primary focus of this research is to bring attention back to the steps that need to be taken before trying to find a way to become pregnant. Inducing puberty and inducing a woman’s first period is part of what makes a woman a woman. As shown in these cases, the transplantation of ovarian tissue can decrease FSH levels while increasing Inhibit B and estradiol levels, ultimately leading to the stimulation of ovarian function. This is a major step in progress for the prepubertal girls who only have one option in fertility preservation.

Applications to Biotechnology
Instead of relying on exogenous hormone administration or embryo cryopreservation, ovarian tissue transplantation shows an alternative approach. By using technology such as the da Vinci surgical robotic system, and allowing hormone levels to increase naturally, transplantation of cryopreserved ovarian tissue shows promise in sustaining fertility through adult life. There is much room for more biotechnology advancements in transplantation.

Acknowledgments
I would like to thank Dr. Ericka for everything she taught me this summer, and the inspiration she has given me to achieve my dreams. I would also like to thank Dr. Chang and Mrs. Winter for sharing their knowledge on us. Also, I could not have had a more amazing experience if it were not for all of my sisters in science. Above all, I would like to thank my parents for everything they have taught and done for me.

References

Figure 1. FSH/estradiol levels at and after transplantation with arrow indicating first menstruation. Adapted from Demeestere et al.1

Figure 2. FSH, estradiol, inhibit B, and AMH levels at and after transplantation. Adapted from Poiriot.2

Figure 3. Levels of FSH following transplantation. Adapted from Ernst. E.2

Figure 4. Adapted from discussion and analysis of research.
DNA shed from endometrial and ovarian cancer can now be detected in pap smears

Elena Medina
Point Loma High School

Objective

It is not recommended for women to get regular screenings for ovarian or endometrial cancers because they can be very invasive and lead to unnecessary stress because of the inaccuracy of the current screening tools, which can oftentimes lead to false negatives or false positives. If women were able to catch these cancers sooner, they could have a lower mortality rate. However, there is a new technique being developed that would allow for screening while a woman is getting a pap smear.

Methods and Materials

In a study done at Johns Hopkins University, 1,658 women were sampled, 1002 of them were healthy and were used as the control variables and the rest of the 656 women had either endometrial or ovarian cancer. To look at the 18 specific genes that previous genome studies confirm are linked to either endometrial or ovarian cancer if mutated, a very precise piece of technology called Safe-Sequencing System (Safe-SeqS) was used. The Safe-SeqS is polymerase chain reaction (PCR) error-reduction machine that allows the use of a unique molecule identifier (UMI) to distinguish rare mutations in the copies of DNA; a primer is also used to show 139 regions and 9392 nucleotide positions that are in the 18 genes being observed. To test the aneuploidy that would show abnormal chromosome numbers another PCR method is used with a single primer on long interspersed nucleotide elements (LINEs), so in the end, you can see which chromosomal arms have abnormalities. To increase the specificity of detecting ovarian cancer specifically 83 women got their plasma tested to look for abnormalities in the chromosomes. Two different brushes were used, the first being the pap brush which could detect 8% of endometrial cancer and 29% for ovarian. The other brush used is the Tao brush which is a thin brush that doesn’t damage the cervix, making sampling the endometrial cavity easy; and it was able to detect 93% of endometrial cancer and 45% of ovarian cancer. It was also shown that looking for ctDNA in a woman’s plasma can increase the specificity of detecting ovarian cancer to 63%. The next step for this technique would be to do another study, but instead of it being a retrospective study change it to a prospective study in order to show how it would work in a clinical setting.

Results

There are currently no good early screening tests for both endometrial and ovarian cancers, which then causes them to have high mortality rates and be the most common female reproductive cancers. If an early screening method was proved to be accurate enough to be used in a clinical setting many women could catch their cancers before they show symptoms, and before the cancer can metastasize. If a woman were to have either endometrial or ovarian cancer DNA from that tumor will spread and can be found on the cervix. The PapSEEK technique uses the sample that is taken from a pap smear during a pap smear and uses the purified DNA from the preservative that is normally used to test for HPV. The DNA is then put into Safe-SeqS which is a PCR error reduction technology and primers allow us to look at 18 specific genes, and look for mutations; also to look for aneuploidy a single primer is applied to LINEs and a PCR method will help detect abnormalities in the chromosomes. Two different brushes were used, the first being the pap brush which was able to detect 81% of endometrial cancer and 29% for ovarian. The other brush used is the Tao brush which is a thin brush that doesn’t damage the cervix, making sampling the endometrial cavity easy; and it was able to detect 93% of endometrial cancer and 45% of ovarian cancer. It was also shown that looking for ctDNA in a woman’s plasma can increase the specificity of detecting ovarian cancer to 63%. The next step for this technique would be to do another study, but instead of it being a retrospective study change it to a prospective study in order to show how it would work in a clinical setting.

<table>
<thead>
<tr>
<th>Stage</th>
<th>Pap brush</th>
<th>Aneuploidy in pap brush</th>
<th>Tao brush</th>
<th>Pap + plasma</th>
</tr>
</thead>
<tbody>
<tr>
<td>Endometrial</td>
<td>81% (382 women)</td>
<td>38% (382 women)</td>
<td>93% (123 women)</td>
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</tr>
<tr>
<td>Early stages</td>
<td>78%</td>
<td>34%</td>
<td>90%</td>
<td>N/A</td>
</tr>
<tr>
<td>Late stages</td>
<td>89%</td>
<td>51%</td>
<td>98%</td>
<td>N/A</td>
</tr>
<tr>
<td>Ovarian Cancer</td>
<td>29% (245 women)</td>
<td>11% (245 women)</td>
<td>45% (51 women)</td>
<td>63% (83 women)</td>
</tr>
<tr>
<td>Early stages</td>
<td>28%</td>
<td>15%</td>
<td>47%</td>
<td>54%</td>
</tr>
<tr>
<td>Late stages</td>
<td>30%</td>
<td>9.3%</td>
<td>44%</td>
<td>75%</td>
</tr>
</tbody>
</table>

Discussion

Since a woman is supposed to get a pap smear every three years, it could be changed to doing a PapSEEK instead so she can get a check on more than just cervical cancer and HPV but checking on the other possible areas for cancer in the woman’s reproductive system. The cost of a PapSEEK is more than just a pap smear, but it would be similar to the cost of a mammogram. This trial showed promising results especially for endometrial cancers, the next step would be to do a prospective trial instead of a retrospective trial to try to mimic its clinical use, but more research needs to be done to be able to distinguish between ovarian and endometrial cancers.

Acknowledgments

I would like to thank Dr. Ericka and Yasmin for helping guide me through my research and helping us all throughout our entire program. I would also like to thank all of our guest lecturers who took time out of their days to come and teach us more about cancer, fertility, and human reproduction.

References

Potential of RNF212, PUMA, and NOXA as Drug Targets Against DNA Damage-Induced Oocyte Apoptosis

Emily Kang
Canyon Crest Academy

Abstract

Due to the aggressive nature of cancer therapy, survivors often face struggles regarding their fertility; however, current fertility preservation options can delay crucial cancer treatment and impact the prognosis of the disease. Chemotherapy and radiation commonly induce DNA damage to oocytes, which can result in apoptosis and diminished ovarian reserve. Thus, there is a need for greater understanding of DSB (double-strand break)-induced apoptosis, which may lead to improved options for cancer patients. In one study performed at UC Davis, RNF212 knockout mice were exposed to 0.35 Gy of γ-irradiation; RNF212−/− oocytes averaged 89% survival, compared to their wild-type counterparts with only 13% survival. After immunostaining for γH2AX, a DNA-damage marker, RNF212−/− oocytes displayed a five-fold reduction in staining compared to the wild-type, suggesting that RNF212 impedes DNA damage repair. RNF212 likely enhances DSB-induced oocyte apoptosis regulated by TAp63. Another study analyzed the mechanism that TAp63-mediated DNA damage-induced oocyte apoptosis uses by examining PUMA and NOXA, both of which are induced by TAp63. PUMA−/− and PUMA−/−NOXA−/− mice were exposed to 0.45 Gy of γ-irradiation; while all primordial follicles were destroyed in wild-type mice, 16% of PUMA−/− oocytes survived, and 52% of PUMA−/−NOXA−/− oocytes survived, with both types of surviving oocytes producing healthy offspring. By targeting RNF212, PUMA, and NOXA, future options may be developed that can maintain genomic integrity and quality of oocytes throughout treatment. Further research must be conducted to ensure that somatic cells are not negatively affected by targeting oocyte apoptosis.

Cancer and Fertility

- Cancer is a major public health concern worldwide and, currently, is the second leading cause of death. From 1991 to 2016, the death rate of cancer fell by 27%, leading to concerns over the quality of life for cancer survivors.

DNA Damage-Induced Oocyte Apoptosis

- Oocyte apoptosis is a normal process that allows organisms to maintain cellular homeostasis by promoting cell turnover and removing cells that are unnecessary, not functional, or potentially dangerous.

Materials and Methods Overview

- WT and RNF212−/− mice at 0.5, 1, 2, 4, 10, and 18 days postpartum (dpp) were irradiated with a single dose of ionizing radiation at 0.35 or 4.5 Gy. Oocytes were stained for γH2AX, a marker of double-strand DNA breaks, and quantified to assess DSBs and quantity of viable oocytes.

Results

- In WT mice, the percentage of oocytes that underwent apoptosis after γ-irradiation increased from 6% to 21% between 0.5 and 2.0 dpp, whereas RNF212−/− mice showed constant low levels of 5% apoptotic oocytes in each cohort.

- A 5-fold decrease in γH2AX staining area was measured in RNF212−/− mice compared to the wild-type (Figure 3).

PUMA and NOXA

- PUMA was found to be highly expressed in oocytes of mice that underwent irradiation, whereas it was not induced in the untreated wild-type cohort (Figure 4).
- Both levels of γ-irradiation led to complete destruction of the primordial follicle pool in wild-type and Trp53−/− mice. After 0.45 Gy γ-irradiation, 19% of 3% of primordial follicles in PUMA−/− mice and 52% of PUMA−/−NOXA−/− mice survived; after 4.5 Gy γ-irradiation, 12% of 1% of primordial follicles in PUMA−/− mice and 94% of 8% in PUMA−/−NOXA−/− mice were protected from apoptosis (Figure 6).
- 13 of 16 Puma−/− females and 9 of 12 Puma−/−NOXA−/− females that were irradiated and mated produced viable offspring, whereas all irradiated WT females tested infertile.

Applications to Biotechnology

- The evidence and findings from the studies described support the use of RNF212, PUMA, and NOXA as drug targets to prevent DSB-induced oocyte apoptosis. As a result, the next steps to be taken are further developments in biotechnology and drug design that are required to develop a safe, effective drug that can target the proposed genes in human patients.

Discussion

- The contrast in percentage of apoptotic oocytes between RNF212−/− and WT mice elucidate the role of RNF212 in leading oocyte apoptosis. The knockout mice were able to sustain a large quantity of their oocytes, even after irradiation.
- Results from γ-H2AX immunostaining support that RNF212 impedes DNA damage repair, and suppressing or knockout the gene may allow patients to maintain their oocytes while also preserving genomic integrity.
- Findings demonstrate that PUMA and NOXA are crucial to TAp63-mediated, DNA damage-induced oocyte apoptosis. By blocking either PUMA or NOXA and PUMA during cancer treatment, fertility may be preserved by preventing oocyte apoptosis, thereby allowing DNA repair and maintaining the ovarian reserve.

Acknowledgements

I would like to extend special thanks to Dr. Ericka Seager-Mitchell for her energy, enthusiasm, and insight throughout the entire program. I would also like to thank Yasmin Khaejennoori, Dr. Chang, Dr. Su, and other presenters for their time and efforts in furthering our growth. Finally, I would like to thank my fellow ROSA sisters and my family for their unwavering support this summer.

References

DECREASING HYPERALGESIA IN ENDOMETRIOSIS: UTILIZING miR-146b AS A BIOMARKER OF DISEASED MACROPHAGES TO INHIBIT IGF-1 VIA LINSITINIB

Emily Tianshi

ABSTRACT

Over 176 million women worldwide suffer from endometriosis, a disease where uterine tissue grows outside of the uterus and causes extreme pelvic pain. (4) The goal of this study is to explore a method of decreasing hyperalgesia. Macrophages stimulate the growth of endometrial lesions. Forster et al. depleted diseased mice of macrophages through liposomal clodronate injections. These mice exhibited similar grooming behavior to healthy mice and had decreased expression of Cox-2, an inflammatory gene, compared to baseline diseased mice, meaning hyperalgesia decreased. Through comparing peritoneal fluid from diseased and non-diseased women, they found diseased macrophages expressed higher levels of the protein IGF-1. Thus, IGF-1 causes extra sensitivity in the nerve cells of lesions during endometriosis. The receptor inhibitor of IGF-1, linsitinib, is an experimental drug, reduced pain levels in diseased mice, quantified through mouse movements (grooming, abdominal retraction, paw withdrawal). (2) However, linsitinib by itself would cause global macrophage depletion. These mice exhibited similar grooming behaviors to healthy mice and had decreased expression of Cox-2, an inflammatory gene, compared to baseline diseased mice, meaning hyperalgesia decreased.

BACKGROUND

Endometriosis
- Uterine tissue grows outside of the uterus
- 176 million women affected worldwide
- 11% of women in the United States affected

Existing Treatments

- Eligard
  - GnRH antagonist, decreases the production of estrogen
  - Forces the body into an artificial menopause
- Side effects: weak bones, hot flashes, painful intercourse, depression, increase in urinary infections, fertility suppression if ovulation is prevented
- Laparoscopic surgery
  - Over 20% of women will not have a decrease in pain
  - Recurs in 50% of women within five years

METHODS

Macrophages In Endometriosis
- Mice with induced endometriosis were depleted of macrophages through liposomal clodronate injections every 48 hours
- Flow cytometry on peritoneal fluids to confirm macrophage depletion

Expression Of IGFR-1 In Diseased Macrophages
- Activated human peripheral blood monocyte-derived macrophages (ABMs) from healthy females with peritoneal fluids (PF) from diseased females to make in vitro endometriosis-associated macrophages (EAMs)

RESULTS

Efficacy Of Linsitinib
- Linsitinib is an experimental drug that inhibits IGFR-1 receptors through paracrine secretion
- Administered every 24 hours
- Insulin and also a insulin receptor inhibitor

miR-146b in Diseased Macrophages
- Genotyping of miR-146b on peritoneal fluid from 74 endometriosis patients and 23 healthy controls

Expression Of IGFR-1 In Diseased Macrophages

- mRNA expression of brain-derived neurotrophic factors (BDNF) was higher in EAMs than other macrophages
- Higher concentrations of IGFR-1 in PF of diseased women than non-diseased
- Concentrations of IGFR-1 correlated to degrees of pelvic pain in women both diseased and non-diseased
- IGFR-1 causes tissue repair and stimulates nerve growth

DISCUSSION

- Pelvic pain is one of the main symptoms of endometriosis.
- Reducing hyperalgesia is a priority to improve patients’ quality of life.
- To summarize, IGFR-1 in macrophages causes pain → linsitinib blocks IGFR-1 receptors → global inhibition should be prevented → diseased macrophages express miR-146b → miR-146b can be upregulated through curcumin

FUTURE WORK

- Upregulation: increasing a cell’s response to a stimulus because of increased receptors
- Does upregulating miR-146b also upregulate IGFR-1 and cause it to be more receptive to linsitinib?
- Integrating linsitinib and curcumin into a coherent drug that only targets IGFR-1 in diseased macrophages

ACKNOWLEDGEMENTS

I would like to thank each and every one of the people I have interacted with in this program: my sisters, special sister Priya, Dr. Ericka, Yasmin, Ms. Winter, Dr. Chang, Dr. Su, and all other doctors and presenters. They have inspired me to pursue excellence in discovery, innovation, and research. ROSA has been a very special experience that encouraged me to not only continue in developing my skills in STEM but to also be an advocate for women around the world.

BIBLIOGRAPHY


ENDOMETRIOSIS: UTILIZING miR-146b AS A BIOMARKER OF DISEASED MACROPHAGES TO INHIBIT IGF-1 VIA LINSITINIB

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FUTURE WORK

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Sulfiredoxin-1 (SRXN1) is Essential for the Reproductive Health of Women with Abnormal Hypothalamic–Pituitary Functions

Joyce Yang
Canyon Crest Academy

Abstract

Gonadotropin-releasing hormone (GnRH) is released by the hypothalamus to stimulate anterior pituitary secretion of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) to regulate fertility. Proper signaling generates reactive oxygen species (ROS). Patients with hyperoxidation (ROS) show less specific responses (NOX/DUOX) to activate mitogen-activated protein kinase (MAPK) 1/3, but excessive ROS can distort GnRH signals and damage cellular structures. Free fatty acids (FFA) have been shown in vitro to distort gonadotropin transcription and induce the unfolded protein response by participating in cell signaling pathways and increasing ROS production1,2, and diet induced obesity has been shown in vivo to increase inflammation in female mice. Various plasma biomarkers of oxidative stress and serum FFA levels are increased with obesity, diabetes, and polycystic ovarian syndrome (PCOS)3,4.

Materials and Methods

To test the effect of chronic exposure to FFAs, 13-week-old female C57BL/6 mice were placed on a 60% high-fat diet (HFD) for 13 weeks. Blood was collected and the mature follicle was ovulated, and the corpus luteum formed. The last stage is diestrus, when the corpus luteus releases progesterone to be reabsorbed as nutrients.

Results

To observe the β-oxidation effects on ROS production in GTL2 cells, cells were cultured with 500 μM 9-β fatty acid oleate (OLA) or MΩA (which is resistant to metabolism) for 3 hours and stained with 5 μM CellROX Green oxidative stress reagent and 1 μM/mL Hoechst 33342. LH and FSH levels were measured by Luminex assay. To observe the effects of FFAs on oocytes, ovaries were fixed in formalin for 48 hours, washed in 70% ethanol, and stained with hematoxylin and eosin to count oocyte/corpus luteum and classify follicles.

In Srxn1 knockdown cells, gonadotropin beta subunit mRNA (Fig. 4) 1. Lhb mRNA but Suppress Fshb mRNA levels at the first and second hours post-treatment between shCTRL and shSrxn1 cells (n ≥ 3).

SRXN1 Reduces PRDX Antioxidant Enzymes

Peroxiredoxins (PRDX) are essential for reducing cellular ROS but can be inactivated by hyperoxidation. Peroxisomal PRDX2 is a regulator of the active cysteine residue (C) of PRDX, which oxidizes cysteine (C-SH) to sulfenic acid (C-SOH). C-SOH stabilizes by reacting with another cysteine residue. C-SOH can be hyperoxidized into sulfenic or sulfonic acid (C-SOOH or C-SOOH) before reduction. Resolution by SRXN1 recycles hyperoxidized C-SOH back to C-SH.

Control and Srxn1 knockdown GTL2 cell lines were cultured with lentiviral shRNA particles for Srxn1 (shSrxn1) and pLK1.1-puro control transduction particles (shCTRL). Both cell lines were cultured with 10 nM GnRH for 7, 8, or 6 hours or untreated for 6 hours. In Srxn1 targeted signaling cascades or ROS, GTL2 cells were pretreated with NOX/DUOX DPI, MAPK 1/3 (U0126 and PD98059), or ROS (NAC) inhibitors or not. To observe gonadotropin beta-subunit gene promoter activity, GTL2 cells were transfected before 5 hour hormone treatments with a pGL3-basic luciferase reporter gene with -1.8 kbp rat Lhb promoters, -398 bp mouse Fshb promoters, or the pGL3-basic luciferase reporter. After hormone treatments, RT-PCR measured cDNA targets, and western blotting measured protein levels. To detect intracellular ROS accumulation, GTL2 cells were transfected with pLW-mCherry2-1 plasmid and ShC1 plasmid or shSrxn1 pLK1.1 plasmid. Cells were stained with CellRox and 1 μM/mL Hoechst 33342 for 30 minutes and fixed in 4% formaldehyde for wide field fluorescence imaging and fluorescence intensity measurement. Flow cytometry detected cellular ROS production.

In Srxn1 knockdown cells, gonadotropin beta subunit mRNA (Fig. 4) 1. Lhb mRNA but Suppress Fshb mRNA levels at the first and second hours post-treatment between shCTRL and shSrxn1 cells (n ≥ 3).

SRXN1 is specifically targeted by GnRH signaling pathways and necessary for proper gonadotropin production. However, this does not explain the whole signaling required for the ovulatory process. Women with obesity, diabetes, and PCOS have abnormal reproductive function. There is a need for improved fertility treatments, as current medications for infertility are expensive and do not promise success. As shown by Sharma et al., chronic exposure to FFAs can disrupt GnRH signaling and impair the estrous cycle and ovulation. Li et al. showed that these phenotypes are responses to FFA induced ROS production. However, this does not explain the whole story between FFAs and fertility, as different FFAs are known ligands that participate in the regulation of gonadotropins 2,5. PRDX have been shown to reduce cellular ROS, but Kim et al. shows that GnRH signaling pathways are necessary for proper gonadotropin expression. However, SRXN1 requires high levels of re-cycled ROS in order to maintain GnRH signaling, potentially losing effectiveness as an antioxidant until there is already irreversible oxidative damage. Even so, the literature shows that SRXN1 is necessary for fertility health and shows potential as a drug target. Acknowledgements

I am extremely grateful for the support of Dr. Ericka Senagor-Mitchell, the ROSA instructors, my ROSA sisters, and Yasmin Khaneji for making ROSA an experience that I will never forget. I would also like to thank my family for allowing me to participate in such a prestigious program and my friends for their support. I am also extremely grateful for Dr. Mark A. Lawton’s guidance as my PI.

References


[Image of the Estrous Cycle]

Figure 1: Illustration of the estrous cycle. Adapted from Ahmed et al. 2016.
Using the Post-implantation Amniotic Sac Embryo (PASE) as an In Vitro Platform to Model Human Amniotic Sac Development

Kaitlin Ordonio

Introduction

The development of the amniotic sac is important because the epiblast and the ectoderm within the amniotic sac have an important job of developing into the embryo itself. If the amniotic sac has defects, a form of infertility is caused because the implanted embryo fails to develop in the amniotic sac. The PASE model only contains amniotic ectoderm-like cells and pluripotent epiblast-like cells. Since the PASE does not have any human organizational form, the PASE stands up against the technical and ethical challenges of harvesting and studying, early human embryo specimens used to study embryology and amniotic sac development.

Abstract

A form of infertility is caused when the implanted embryo fails to develop within the amniotic sac. The PASE was created and tested to see if the model was viable. It was a viable amniotic sac replica to solve technical and ethical challenges of harvesting and studying early human embryos. One study showed the development of a biomimetic 3D culture system where hPSCs were plated as single cells onto different densities of Gelatin-B. Results showed similar human amniotic ectoderm-epiblast tissue patterning only if cell plating density was in the intermediate range of 30,000-50,000 cells cm⁻². Another study used immunofluorescence analysis to characterize cell fate. The results from staining showed that the columnar side of the amniotic sac is composed of cells that contain the pluripluripotency markers OCT4, NANOG, and SOX2. These same markers have been seen exclusively in the embryonic disc of post-implantation monkey embryos. Immunofluorescence analysis on models of PASE to see if there were patterns of BMP1 signaling. In day 5 PASE, there is EMT which is a phenotype associated with PS-initiation found in Carnegie stage 6 embryos. The results of this study show similar PS-initiation among both human amniotic sacs and PASE. During another study on embryogenesis in mice, BMP-SMAD signaling also played an important role in morphogenesis. Results showed that there is no BMP2 or Smad5. There are defects in both amniotic and embryonic patterning. Staining of SMAD1/5, an activator of BMP-SMAD signaling was used in immunofluorescence analysis on models of PASE to see if there were patterns of BMP-1 in the PASE tissue.

Methods

Experiment 1: Analyzing Cell Plating Density in Gelatin Beds

The PASE was made from human pluripotent stem cells (hPSCs). (Figure 2) hPSCs are plated as single cells at different cell plating densities onto a thick, soft gel bed of Gelretx. Cell plating density was then examined revealing a clear dependence on initial cell plating density. The different plating densities were intermediate range (30,000-50,000 cells cm⁻²), highest plating density (70,000 cells cm⁻²) and low plating density (20,000 cells cm⁻²).

Experiment 2: Immunofluorescence Analysis to Characterize Cell Fate

The columnar side of the amniotic sac is composed of cells that contain the pluripotency marker OCT4, which is not seen in the squamous side of the model. Immunofluorescence analysis with co-staining of OCT4, NANOG and SOX2 were used to ensure that the amniotic ectoderm (squamous side) only contained HOECHST, which are nucleic acid that were used for pluripluripotency markers such as NANOG, OCT4 and SOX2. HOECHST was used for counterstaining cell nuclei.

Experiment 3: Immunofluorescence Analysis of OCT4 on PS-Initiation

Through immunofluorescence analysis of the markers OCT4, NANOG and SOX2, it showed cells exit from pluripotency and suggested a non-neuroectodermal differentiation of these cells. These cells went through epithelial-to-mesenchymal transition, EMT, which is a phenotype associated with primitive streak (PS) initiation. Next, the transcription factor associated with PS development, BRACHURY (BRA) was used to analyze PASE with a PS-like phenotype. In the experiment they identified three stages of PASE development that would be tested for the marker BRA. 173 models of PASE were used in this experiment.

Experiment 4: BMP-1 in Embryonic Patterning

A previous study of the PASE showed activated BMP-1 signaling during the development of PS, as ectoderm-like cells. Furthermore, a study of embryogenesis in mice showed that BMP-1 signaling played a novel role in the amniotic sac development. In the study, it was revealed that BMP-1 signaling may play an important role in maintaining the stable asymmetric amniotic sac-epiblast tissue pattern during PASE development.

Results

Discussion

Through the PASE, hPSCs can model multiple human embryonic events, such as morphogenesis, cell fate patterning of the amniotic sac, posterior PS formation and BMP-1 signaling seen in embryonic discs and tissues of monkey embryos. From these experiments, it is noted that there is potential for hPSCs to create the first embryoid model for studying post-implantation amniotic sac development, which is drastically different from amniotic sacs belonging to mice. The future goals of the PASE is to understand the molecular mechanisms underlying the asymmetric activation of BMP1-SMAD in PASE because this area seems unknown. Another goal is to examine the PASE model over 5 days in culture, because all experiments were terminated in the PASE before embryos reached 14 developmental days. Although there are some limits to this model of the amniotic sac, it is still the first hPSC-based embryoid model and it gives investigators and scientists a big leap towards advancing human embryology, reproductive medicine and infertility caused after implantation.

Application to Biotechnology

Human pluripotent stem cells are cells that can self-replicate, derived from human embryos or human fetal tissue and can develop into cells and tissues of the ectoderm, mesoderm and endoderm. These hPSCs can potentially produce any cell or tissue the body needs to repair itself. Because of this advantage, hPSCs are becoming very useful to new medical solutions for workers in the Pharmaceutical and Human embryology. hPSCs also have potential that patients could receive transplants of tissue and human without facing problems like tissue rejection and tissue matching. The PASE model is just one example of hPSC potential to move towards advanced biomedical solutions. hPSCs could advance reproductive medicine and infertility solutions.

Acknowledgements

First, I would like to thank Dr. Ericka Senegar-Mitchell for all the guidance and support she has given. I would like to thank Dr. Chang and Ms. Patricia Winter for organizing this program and giving us amazing opportunities. I also want to thank my science teachers at OLP that guided me along this path, for my love of science grew and so did my knowledge. Furthermore, I want to thank my parents for supporting me. I want to thank Yasmine Khajenoori for always being there and helping me and my ROSA sisters. Lastly, I want to thank Ayesha Adam-Mir who was always there for me when I needed help.

References


Individual responsiveness, an incomplete understanding of the ovaries, and varying infertility causes leave controlled ovarian stimulation (COS) challenged by low success rates. In hundreds of conventional GnRH-antagonist cycles, 34-38% of patients experience premature LH surge. This manuscript aims to define the efficacy of Progesterone-Primed Ovarian Stimulation (PPOS) compared to conventional GnRH analogs in blocking the LH surge, offering women better control of the ovulatory production process and thus increasing the probability of completing in vitro fertilization. The study was conducted in a randomized clinical trial with two groups. The study group received a daily progesterone pill and the control group received GnRH antagonist subcutaneous injections on Day 8 of stimulation, with both treatments taking place after 12 days. Both groups were ultimately tracked for their mature oocytes to directly measure the effectiveness of progesterone in stopping LH surge incidence and produce higher numbers of oocytes for retrieval. The Wang et al. trials found that only 3.0% of their poor responders receiving PPOS had an incidence of premature LH surge versus 8.0% of GnRH antagonist patients. The PPOS and GnRH antagonist groups were not significantly different in their mature oocyte counts. However, the use of PPOS for oocyte retrieval may be viable for women suffering from fertility issues as a strong alternative with potentially lower costs, being an overall simpler procedure. PPOS methods have potential research avenues in comparing the efficiency of different types of progestins available and analyzing if any type suits specific patient populations.

**Experiment 1:**

1. **Objective:**
   - To compare the efficacy of PPOS with GnRH analogs in blocking the LH surge.
   - The main outcome measure was the number of mature oocytes retrieved from each group.

2. **Methods and Materials:**
   - Patients aged 41 years or older with 1.00 mg/ml of AMH in their first of second IVF/ICSI cycle. Patients with greater than 15 follicles or over 0.038 mg/ml of AMH were given GnRH agonists.

3. **Results:**
   - The primary outcome was the number of mature oocytes retrieved from each of the two groups.
   - Ultrasonography tracked follicular growth and regular blood samples to check levels of E2, LH, and P4. Statistical analysis was carried out through StatXact 6.0.

**Experiment 2:**

1. **Methods and Materials:**
   - The study involved 67 PPOS and 90 GnRH antagonist women who all had known PCOS (polycystic ovary syndrome).
   - The control group received FSH and HMG in varying dosages as each PCOS patient’s sensitivity was unique.
   - Follicles were injected with 0.25 mg of GnRH antagonist injections beginning to be administered daily.
   - The study group received 150 to 225 FSH/human menopausal gonadotropins injections along with a daily oral 10mg MPA (medroxyprogesterone) pill after Day 3 of stimulation.

2. **Results:**
   - The final trigger was induced by 0.25mg injection of decapetepl 2000 IU HCG after three dominant follicles were greater than 18mm.
   - The oocytes were ready for retrieval after 36 hours of the trigger and were extracted with the aspiration needle with ultrasound guidance. Statistical analysis was carried out using SPSS v16.0.

3. **Conclusions:**
   - PPOS women are randomized into two groups.
   - Both groups have a significantly higher number of retrieved oocytes compared to the GnRH antagonist group, suggesting that PPOS may be a promising strategy to improve IVF outcomes.

**Acknowledgments:**

1. Thank you to you and your colleagues for your hard work and dedication to the field of fertility.
2. We appreciate the financial support provided by the National Institutes of Health.
3. We also acknowledge the assistance of our technicians and laboratory staff.

**References:**

The immunosuppressive and recurrent behavior of lactate from hypoxic tumor cells is significant in pancreatic adenocarcinoma (PDAC), which exhibits high relapse rates. Monocarboxylate transporters, or MCTs, which are responsible for lactate flux in hypoxic tumor cells, including those of PDAC, are not present in healthy pancreatic cells. Thus, inhibiting MCTs in PDAC offers a non-toxic therapeutic approach to cancer recurrence caused by lactate metabolism of hypoxic tumor cells. This novel approach raises excitement over the potential of a minimally invasive and fertility-preserving treatment to PDAC that attacks recurrence. The objective of this research is to analyze multiple studies to determine an inhibitor that allows for these potentials to become a reality.

Methods and Materials

Three studies utilizing different approaches to determine an inhibitor of MCT1 and MCT4 were compared in this meta-analysis. The Draoui et al study synthesized direct inhibitors of MCT1 and MCT4, whereas the Wilson et al study and Voss et al evaluated inhibitors of CD147, an enzyme essential to the assembly of MCT1 and MCT4.

Draoui et al study: researchers inferred that the best way to inhibit a monocarboxylate transporter was to design a molecule that had monocarboxylate-containing coumarins in the scaffold. 23 monocarboxylic acid derivatives were synthesized using the classical Vilsmeier–Haack reaction and cyclisation with Medium acid chloride to result in the final compounds. A primary assay was performed to identify compounds which selectively inhibited tumor cell proliferation in culture environments but displayed non-toxicity in glucose rich environments (experimental cells derived from human cervix carcinoma cell line SiHa). A secondary assay measured [14C]-lactate uptake by SiHa cells on a 12 minute time frame and a tertiary assay measured inhibition of [14C]-lactate influx. The researchers then evaluated the IC50 in vivo and in vitro profiles of c19 alongside performing a structure-activity relationship (SAR) analysis. In order to exclude major anticoagulant side effects, the researchers then tested mortality of c19 in comparison with Warfarin (reference compound with known anticoagulant effects).

Wilson et al study: rabbit erythrocytes were treated with pCMBS to determine whether pCMBS targets CD147. The necessary data was obtained by treatment of cells with the bifunctional organomercurial reagent fluoresein dimercury acetate that caused oligomerization of CD147.

Voss et al study: 727 drugs were screened via a cell-based MCT-Basigin interaction assay utilizing a synthetic Renilla luciferase (Rluc) protein-fragment-assisted complementation-based Basigin interaction assay. 8 A classical Vilsmeier–Haack reaction was used in the synthesis of MCT1 and MCT4 inhibitors. A primary assay was performed to identify compounds which selectively inhibited tumor cell proliferation (experimental cells derived from human cervix carcinoma cell line SiHa). In lactate medium, 10μM of compound 19 (c19), a 7-alkylamino 3-carboxycoumarin, resulted in SiHa cell proliferation of less than 20% cell density, whereas CHC, the reference compound, had 50% cell density. Additionally, IC50 (compound concentration to reduce lactate uptake by 50%) and EC50 (compound concentration to reduce cell growth by 50%) and EC50 (compound concentration to reduce cell proliferation by 50%) of c19 were 0.059μM and 0.22μM, respectively, compared to 43.5μM and 10.7μM for CHC. Though other substances of MCT1 and MCT4 were identified, c19 is a promising candidate due to its low toxicity in vitro ADME and in vivo PK properties along with no anticoagulant side effects. Its ability to minimize toxicity without compromising efficacy makes c19 a viable solution to pancreatic cancer recurrence.

Results

Draoui et al study: In lactate medium, 10μM of compound 19 (c19), a 7-alkylamino 3-carboxycoumarin, resulted in SiHa cell proliferation of less than 20% cell density, whereas CHC, the reference compound, had 50% cell density. Additionally, IC50 (compound concentration to reduce lactate uptake by 50%) and EC50 (compound concentration to reduce cell growth by 50%) and EC50 (compound concentration to reduce cell proliferation by 50%) of c19 were 0.059μM and 0.22μM, respectively, compared to 43.5μM and 10.7μM for CHC. Though other substances of MCT1 and MCT4 were identified, c19 is a promising candidate due to its low toxicity in vitro ADME and in vivo PK properties along with no anticoagulant side effects.

Wilson et al study: Site-directed mutagenesis of CD147 suggested HC(2)147 strongly inhibited MCT1-CD147 as well as MCT4-CD147 interaction, preventing lactate transport into rabbit erythrocytes.

Voss et al study: Using a cell-based drug-screening assay, Acriflavine (ACF) was identified as an inhibitor of Basigin (also known as CD147) and MCT4 interaction. The in vitro experiments indicated that ACF appears to primarily disrupt the interaction between MCT4 and Basigin but not the interaction between MCT1 and Basigin. The researchers found that while hypoxia induced MCT4 expression by over 60-fold, ACF treatment significantly inhibited this induction to 20-fold, an over 60% reduction. ACF was found to have an IC50 of 5 μM.

Discussion

As per the results, each of the three candidates-c19, pCMBS, and ACF—have a variety of positive and negative traits. c19 had approximately 100 times lower IC50 than ACF, implying that hypoxia in PDAC would be far more receptive to c19 than ACF. Though c19 was tested in vivo in mice via intraperitoneal injection, there is no data on c19 behavior specific to the pancreas. pCMBS inhibits both MCT1 and MCT4 interaction with CD47, but there is no literature on its potential anticoagulant side effects or utility in cancer, specifically hypoxic tumor cells. Finally, ACF demonstrates high efficacy when initially inhibiting MCT4 and CD147 interaction, but there is little published research on its anticoagulant side effects or data on its behavior in the pancreas. It should also not be ignored that the glioblastoma cells in the ACF experiments gradually developed resistance to ACF, bringing into question the long-term efficacy of ACF. However, if ACF proves to have minimal negative side effects, there is potential for ACF to be used in tandem with c19 in order to maximize inhibitory effects on both MCT1 and MCT4.

References

V. Reference...