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

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Objective

Even with growing knowledge and research of fertility preservation techniques and treatments for known pathologies related to infertility, there still remains a significant gap in the causes and implications of unexplained infertility and miscarriages. G-CSF, granulocyte stimulating factor, shows potential in its ability to strengthen the endometrium in order for a female to establish and maintain a pregnancy. In a series of three trials, researchers test G-CSF's effect on immune response to implantation, thin endometrium, unexplained infertility, and IVF success.

Abstract

G-CSF is a colony stimulating factor that targets leukocytes and produces stem cells required for establishing and maintaining a pregnancy.[5] Through research development, it is becoming a promising new tool for women with unexplained primary recurrent miscarriages (RM) and biochemical pregnancies due to implantation failure.[7] Unexplained infertility continues to vex reproductive researchers and clinicians. However, there is hope that G-CSF will increase pregnancy rates through improving the quality and thickness of the endometrium and stopping the immune response that disables an embryo's ability to implant.[4] A trial was conducted with 68 women with unexplained primary RM where researchers randomly administered G-CSF (1 microg/kg/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. The G-CSF was administered in hopes of allowing women with RM or repeated IVF failure to develop the response of the ovary to the pharmacologic stimulatory treatment. In the group treated with G-CSF, 29 out of 35 (82.8%) women experienced little to no pregnancy complications and delivered a healthy baby. Whereas in the placebo group, just 16 out of 33 (48.5%) had a healthy pregnancy and a live birth.[6] With further knowledge and application of G-CSF, researchers can not only improve embryo implantation and ovarian function but also stimulate endometrial thickening and pathologies relating to the endometrium.[3] This will help solve the mystery of unexplained infertility.

Methods and Materials

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 secretion, which mediates the activation and maintenance against extracellular threats, along with the activation of T regulatory cells. These two factors may influence crucial gene expression in the endometrium, including local immune modulation, vascular remodeling of the endometrium, and cellular adhesion pathways. In females who lack the proper immune response during implantation (the endometrium rejects the embryo), G-CSF could target these responses for establishing and maintaining pregnancy.

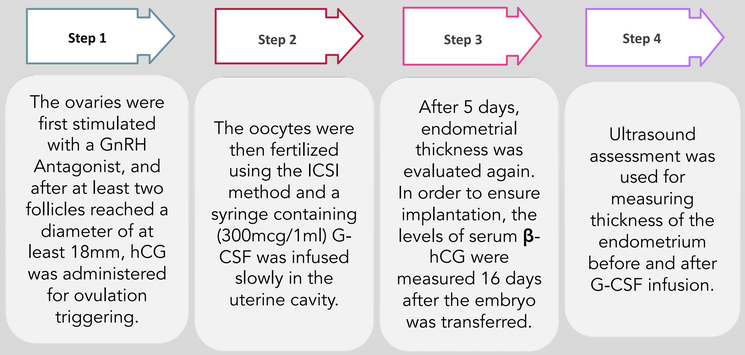

Figure 1: Steps taken in determining the influence of G-CSF on immune response and thin endometrium.[5]

Methods and Materials (continued)

B. Study 2: RM/unexplained infertility

The 68 patients involved in trial 2 were randomly assigned to the two arms of the study, one to the G-CSF treatment and the other to a placebo administration. A trial was conducted with 68 women with unexplained primary RM.

Experimental Group: with application of G-CSF (n=35)	Placebo group (n=33)
Researchers randomly administered G-CSF (1 microg/kg/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.

Figure 2: Experimental and Placebo group in Study 2 (effect of G-CSF on RM and infertility).[6]

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.[6]

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 oocytes collection, 300 mg G-CSF was administered by slow transcervical intrauterine 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.[4]

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.[5]

		Conceived (n = 3)	Not conceived (n = 12)
Endometrial thickness at day of G-CSF infusion (mean ± SD)	3.6mm ± 0.98	3.6mm ± 1.5	3.4mm ± 0.87
Endometrial thickness at day of embryo transfer (mean ± SD)	7.120mm ± 0.84	7.5mm ± 1.4	7.mm ± 0.71
Δ endometrial thickness (mean± SD)	3.53mm ± 0.88	4.2mm ± 1.3a	3.6mm ± 0.72b

Figure 3: Endometrial thickness in patients before and after treatment with G-CSF (n = 15)[5]

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.[6]

	G-CSF	Placebo	*P*-value
Number of live births (%)	29 (82.8)	16 (48.5)	0.0061
Number of miscarriages (%)	6 (17.2)	17 (51.5)	0.0061
Gestational week of miscarriage (mean ± SD)	6.0 ± 1.1	6.2 ± 1.0	0.6989
Newborn weight (g, mean ± SD)	3050 ± 220	3125 ± 240	0.3098

Figure 4: Results of the study in patients treated with G-CSF and controls (placebo) [6]

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.[4]

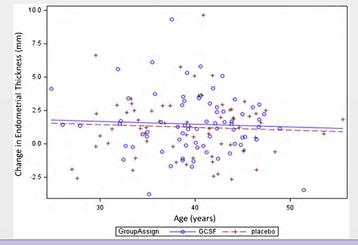

Figure 5: Change in endometrial thickness in mm from initial day of hCG until day of transfer: C G-CSF; + placebo. Mean change solid line (G-CSF); dashed line (placebo).[1]

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 with 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 still troubling 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 Senegar-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 in by each and every one of the presenters throughout the academy. Id especially like to thank Dr. Irene Su and Dr. Jeffery Chang for expanding my love and curiosity for medicine. To all my ROSA sisters and big sister in science, Yasmin, for their continued support. Finally, Id like to thank Ms. Patricia Winter and the Oncofertility Consortium for making this program possible.

References

1. Barad, D. H., Yu, Y., Kushnir, V. A., Shohat-Tal, A., Lazzaroni, E., Lee, H., & Gleicher, N. (2014). A randomized clinical trial of endometrial perfusion with granulocyte colony-stimulating factor in in vitro fertilization cycles: Impact on endometrial thickness and clinical pregnancy rates. Fertility and Sterility,101(3), 710-715. doi:10.1016/j.fertnstert.2013.12.016
2. Li, J., & Chen, Y. (2017). The Effect of G-CSF on Infertile Women Undergoing IVF Treatment. Reproductive Immunology: Open Access, 02(01). doi:10.21767/2476-1974.100033
3. Liu, K. E., Hartman, M., Hartman, A., Luo, Z. -., & Mahutte, N. (2018). The impact of a thin endometrial lining on fresh and frozen–thaw IVF outcomes: An analysis of over 40 000 embryo transfers. Human Reproduction, 33(10), 1883-1888. doi:10.1093/humrep/dey281
4. Mostafa, F. (2017). Effect of Intrauterine Infusion of Granulocyte Colony Stimulating Factor on IVF Outcomes in Infertile Women. Journal of Gynecological Research and Obstetrics, 011-014. doi:10.17352/jgro.000030
5. Naghshineh, E., Eftekhar, M., & Khani, P. (2018). Role of granulocyte colony-stimulating factor in human reproduction. Journal of Research in Medical Sciences, 23(1), 7. doi:10.4103/jrms.jrms_628_17
6. Scarpellini, F., & Sbracia, M. (2009). Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: A randomised controlled trial. Human Reproduction, 24(11), 2703-2708. doi:10.1093/humrep/dep240
7. Vollenhoven, B., & Polyakov, A. (2009). Faculty of 1000 evaluation for Use of granulocyte colony-stimulating factor for the treatment of unexplained recurrent miscarriage: A randomised controlled trial. F1000 - Post-publication Peer Review of the Biomedical Literature. doi:10.3410/f.1165442.630076

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

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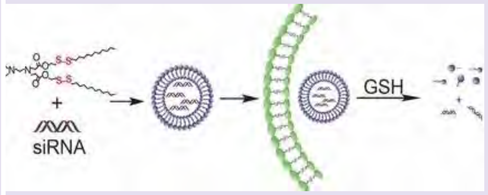
Abstract

Endometriosis, a disease in which endometrial tissue grows outside of the uterus, affects 10% of women in the United States, causing damage to pelvic organs, intense menstrual pain, and infertility. Current treatments include hormonal supplements to induce anovulation and surgical removal of lesions and growths.[1] Recent developments in delivering genetic therapy show promise as a method of less invasive and less obstructive treatment; liposomal and lipid-peptide 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 grafted chitosan micelles (CSO-SA) and a pigment epithelium derived factor (PEDF) plasmid were combined as a method of genetic inhibition for angiogenesis. Intravenous injection led to not only 48.79% decrease in lesion volume but also a significantly increased apoptosis index of 11.00 ± 6.83 as compared to the control index of 5.25 ± 1.91.[4]. Similarly, transcription growth factor-beta (TGF-β), like VEGFA, microRNA-451a, and other genes that influence cytokine pathways causing cell multiplication, was found to be overexpressed in ectopic endometrial tissues; TGF-β is a migratory factor causing invasion and migration of approximately 100 more endometrial stromal cells than cells of control groups. TGF-β activates extracellular signal-regulated kinase (ERK) and mitogen-activated protein kinase (MAPK) signaling pathways.[2,3,5] Use of lipid-based markers and nanoparticles as protein-carriers has shown high efficiency as well, nanoparticles being able to alter up to 80% of gene expression in target tissue.[6] With the efficiency of delivery methods for genetic material and the targeting of ectopic cells, genetic therapies show potential for stopping the vascularization and migration of endometriotic tissue by inhibiting expression of the culprit genes, transcription factors, and miRNAs.

Figure 1: Lipidoids and the protection of siRNA against GSH in the blood; Lipidoids as an effective carrying method for siRNA Adapted from Bedin et. al[2]



Introduction

Ectopic tissue, contrasting with eutopic, is endometrial tissue that grows in pelvic areas beyond reproductive organs from the parametrium to peritoneal tissues. Different types of endometriosis are characterized by the intensity of the implantation of tissue, their receptivity to progesterone and estrogen, and the areas in the pelvis in which ectopic lesions proliferate. The tissue, in response to normal cycling or hormones associated with the menstrual cycle, sheds and causes internal bleeding, intense menstrual pain, and infertility. Treatments are just as and painful, with no guarantee of relief; hormonal treatments can alter a patient's regular cycle with no guarantee of pain relief, leaving painful menstruation. Surgical treatments are invasive and can leave further scarring of tissue, with no guarantee tissue will not regrow.[1] Such proliferation is caused by characteristics similar to those of cancerous cells in the sense that apoptosis appears to be inhibited and certain regulatory pathways show altered genetic expression.[4] With the receptivity of endometrial tissue to the lipid-based hormone estrogen and recent developments of lipid-based particles as carriers and protectants for gene-editing materials, use of lipid carriers of genetic material may provide a less invasive, more permanent therapy for endometriosis as compared to surgery and hormonal supplements.[1,2,3,4,7,9]The objective of this research is to determine the efficacy of lipid-particles in carrying genetic material to reduce the proliferation or growth of ectopic tissue in endometriosis.

Methods

Experiment 1: *Gene therapy of endometriosis introduced by polymeric micelles with glycolipid-like structure*

A polymeric micelle was formed using pigment epithelium derived factor (PEDF) and lipid grafted chitosan micelle (CSO-SA); PEDF was carried in an Escherichia *coli* DH5a plasmid, cloned as a pCR-II vector. Chitosan was deacetylated and washed among a number of chemical solutions to form micelles, the concentration of which was measured through pyrene fluorescence. A CSO-SA/PEDF complex was created was prepared through mixing purified CSO-SA and 500 µg/ml PEDF solution. The resulting nanoparticles were distributed into the model organisms. Rats maintained under standardized conditions had endometrial lesions surgically induced. Endometrial tissue was collected from a 1 cm segment of the uterine horn; the tissue was then segmented, and sutured into the peritoneum of the abdomen in 5 by 5 mm^2 segments. Post recovery, the nanoparticle micelles, now labeled with fluorescein isothiocyanate (FITC) and placed in a buffer solution containing 1000 µg 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 PEDF injected as well and received CSO-SA/PEDF treatment.[4]

Experiment 2: *Enhanced Intracellular siRNA Delivery using Bioreducible Lipid-Like Nanoparticles (See Figure 1)*

Bioreducible lipidoids (lipid-like nanoparticles) were synthesized. To test the siRNA delivery capabilities, a breast cancer cell line was transfected with siRNA targeting an induced green-fluorescent protein (GFP) gene. siGFP effectiveness was measured through cytometry analysis and fluorescence of treated cells. One group of cells was treated with naked siGFP, and others with lipidoid/siGFP, groups of which were separated by amine number and number of carbon atoms in the tail of the lipidoid. A last group was treated with a commercial transfectant, Lipofectamine 2000. A gel retardation assay, dynamic light scattering (DLS), and transmission electron microscopy (TEM) were also performed to observe the qualities of the nanoparticle lipidoids, seperated into groups treated with glutathione (GSH) and those not. Human cervical carcinoma cells (HeLA) and murine breast cancer cells (4T1) were transfected to test the efficacy of delivery of siRNA through lipidoids, results of which were measured in percent cell viability.[9]

Results

Experiment 1: The CSO-SA/PEDF complex condensed the DNA successfully, protecting the plasmid from degradation when facing UV rays are compared to the bare plasmid which degraded in 5 minutes, proving stability in vivo. FITC labeled CSO-SA/PEDF presence in cysts was successfully observed through fluorescence. Mean lesion volumes amongst the three groups (control, CSO-SA/PEDF, and danazol) showed no significant difference (P>0.05) initially, but after two weeks the inhibition of cyst growth using CSO-SA/PEDF was 48.79%, with a mean volume of 34.79 ± 19.70 mm3 as compared to the initial volume of 54.38 ± 14.49 mm3, observed in Figure 2. The CSO-SA/PEDF micelle treated group also had lessened gland structure and a suppressed gland structure, observed in Figure 3. Microvessel density is decreased in the CSO-SA/PEDF treated group at 7.86 ± 3.13 as compared to 14.88 ± 7.45 of the control group. The experimental group had an apoptotic index of 11.00 ± 6.83 compared to the control group index of 5.25 ± 1.91, revealing a significant difference. (See Figure 4)[4]

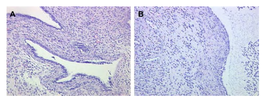


Figure 3: Micrographs displaying abundant epithelial cells and glands in control group (A) as compared to reduced gland structure in experimental CSO-SA/PEDF treated group (B) Adapted from "Gene Therapy of Endometriosis Introduced by Polymeric Micelles with Glycolipid-like Structure." [6]

Table 1: MicroRNAs Observed in Endometriotic Tissue and Corresponding Effects of Regulation or Inhibition[5,6,8,12,19]

MiRNA	Role Upon Observation	Cell Type Tested In	Expression, Inhibition, or Regulation	Effect
MicroRNA 200b	Regulation of KLF4 and ZEB1, ZEB2 transcription factors	Human	Overexpression	Decreased proliferation of cells,
MicroRNA 451a	Increased cell growth and proliferation (initially overexpressed)	C57BL/6J wild-type mice	Inhibition	Decrease in lesion volume,

Results

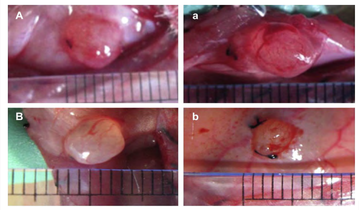


Figure 2: The lesion size before of the control (A) and CSO-SA/PEDF treated rats (B) vs. after two weeks (a,b respectively) Adapted from "Gene Therapy of Endometriosis Introduced by Polymeric Micelles with Glycolipid-like Structure." [4]

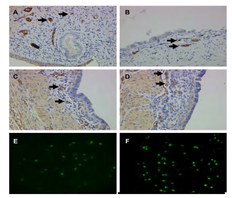
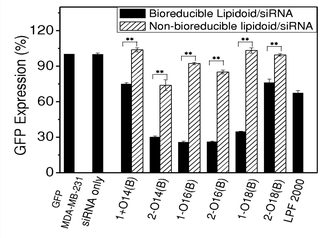


Figure 4: Stained vascular tissue in the control (A) is increased compared to CSO-SA/PEDF (B), apoptotic cells in control (E) less than those of treated cells (F) Adapted from "Gene Therapy of Endometriosis Introduced by Polymeric Micelles with Glycolipid-like Structure." [4]

Experiment 2: Bioreducible lipidoid carriers of siRNA showed decreased GFP expression in the breast cancer cell line of at least 15% and at most about 70% as compared to the expression in the control groups (see Figure 5). TEM imaging revealed lipidoids underwent degradation in the presence of GSH. Increasing sizes of the complexes (in nM) revealed greater efficacy, with GFP expression reduced to under 30% using 8 nM particles. Delivery in HeLA and 4T1 cells revealed inhibited proliferation of cancer cells (dependent on siRNA dose), varying based on the structure of the lipidoid complex. Overall cell viability using siRNA carrying lipidoids revealed greater cell viability than the LPF2000 control.[9]



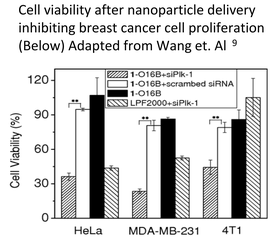


Figure 5: GFP expression altered by different structured lipidoids in breast cancer cell lines (Left)[9]
Cell viability after nanoparticle delivery inhibiting breast cancer cell proliferation (Below) Adapted from Wang et. Al [9]

Discussion

PEDF expresses glycoprotein 50-KDa, a compound known to inhibit angiogenesis. The glycolipid-complex provided by chitosan protected the genetic material in the PEDF plasmid, forming a complex that protected genetic material due to the lipid-based structure. This effectively target ectopic cells within the model organisms, inhibiting cyst growth and vascularization while not affecting the reproductive organs, showing no cytotoxicity as well as effective targeting of ectopic sites. Cells effectively internalized the micelles are the alkyl chains adhered to the membrane and the liposome was carrying genetic material was engulfed. Decreased vascularization and increased apoptosis indicate significant reduction of proliferation of lesions.[4] The success of the lipidoid complexes in reducing GFP expression in breast cancer cells and release genetic material has shown that lipid structures can be optimized to deliver genetic material effectively. Overall, these three studies reveal that ectopic tissue can be targeted effectively with lipid-based particles carrying genetic information; with several genes overexpressed, genetic therapy through lipid-nanoparticles is shown to effectively regulate gene expression in endometriosis.[8]

Table 2: Genes Observed in Endometriotic Tissue and Corresponding Effects of Altered Expression[5,6,8,12,19]

Gene	Role Upon Observation	Cell Type Tested In	Expression or Regulation	Effect
TGF-β	Promoting ERK and MAPK pathways	Human	Overexpression	Migration, inflammation, invasion, resistance to apoptosis
VEGFA	Promotes angiogenesis and migration of endothelial tissue	Wistar Rat	Downregulation	Shrunken endometrial implants in test subjects, reduction of microvessels in lesions

Relevant Applications to Biotechnology

Evaluation of the efficiency of lipid nanoparticles and peptide-lipid conjugates as carriers of genetic therapies for endometriosis provides the potential for a new drug therapy for endometriosis. Previously, hormonal treatments were the only non-invasive treatment but would affect both eutopic and ectopic tissue.[1] With new developments in observing pathways of certain transcription factors upregulated in endometriosis, siRNAs can be developed to target these factors and block pathways that cause cell proliferation and angiogenesis, the causes of damage to reproductive organs, recurrence post-surgery and immense menstrual pain.[1, 4] Genetic material protected within lipid nanoparticles are showing efficacy through both intravenous and oral administration; future development of silencing technologies using lipid factors to target ectopic lesions and protect genetic material provide for genetic therapies that can be administered orally, and could potentially prevent the progression of endometriosis from early diagnosis. Women could prevent the damage that causes infertility, and take a drug with these factors while having normal, less painful menses. Overall, there is potential for a new medical intervention in preventing damage and pain associated with endometriosis while preserving eutopic endometrium. With some genetic overexpression observable in blood and serum, a noninvasive therapy could be instilled.

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References

1) Agarwal, Neha, and Arulselvi Subramanian. "Endometriosis – Morphology, Clinical Presentations and Molecular Pathology." Journal of Laboratory Physicians 2, no. 1 (2010): 1–9. https://doi.org/10.4103/0974-2727.66699.
2) Bedin, Alessandra, Raul C Maranhão, Elaine R Tavares, Priscila O Carvalho, Edmund C Baracat, and Sérgio Podgaec. "Nanotechnology for the Treatment of Deep Endometriosis: Uptake of Lipid Core Nanoparticles by LDL Receptors in Endometriotic Foci." Clinics 74 (2019): e989. https://doi.org/10.6061/clinics/2019/e989.
3) Eggers, Julia C., Valentina Martino, Rolland Reinbold, Sebastian D. Schäfer, Ludwig Kiesel, Anna Starzinski-Powitz, Andreas N. Schüring, Björn Kemper, Burkhard Greve, and Martin Götte. "MicroRNA MiR-200b Affects Proliferation, Invasiveness and Stemness of Endometriotic Cells by Targeting ZEB1, ZEB2 and KLF4." Reproductive Biomedicine Online 32, no. 4 (April 2016): 434–45. https://doi.org/10.1016/j.rbmo.2015.12.013.
4) "Gene Therapy of Endometriosis Introduced by Polymeric Micelles with Glycolipid-like Structure." Biomaterials 33, no. 2 (January 1, 2012): 634–43. https://doi.org/10.1016/j.biomaterials.2011.09.077.
5) Liu, Zhihong, Lisha Yi, Miaomiao Du, Guifang Gong, and Yali Zhu. "Overexpression of TGF-β Enhances the Migration and Invasive Ability of Ectopic Endometrial Cells via ERK/MAPK Signaling Pathway." Experimental and Therapeutic Medicine 17, no. 6 (June 2019): 4457–64. https://doi.org/10.3892/etm.2019.7522.
6) Puri, Anu, Kristin Loomis, Brandon Smith, Jae-Ho Lee, Amichai Yavlovich, Eli Heldman, and Robert Blumenthal. "Lipid-Based Nanoparticles as Pharmaceutical Drug Carriers: From Concepts to Clinic." Critical Reviews in Therapeutic Drug Carrier Systems 26, no. 6 (2009): 523–80.
7) Taylor, Menghui Li, Yuping Zhou, Hugh S. "MiR-451a Inhibition Reduces Established Endometriosis Lesions in Mice - Menghui Li, Yuping Zhou, Hugh S. Taylor,." Reproductive Sciences, July 28, 2019. https://journals.sagepub.com/doi/10.1177/1933719119862050.
8) Wang, Ming, John A. Zuris, Fantao Meng, Holly Rees, Shuo Sun, Pu Deng, Yong Han, et al. "Efficient Delivery of Genome-Editing Proteins Using Bioreducible Lipid Nanoparticles." Proceedings of the National Academy of Sciences of the United States of America 113, no. 11 (March 15, 2016): 2868–73. https://doi.org/10.1073/pnas.1520244113.
9) Wang, Ming, Kyle Alberti, Antonio Varone, Dimitria Pouli, Irene Georgakoudi, and Qiaobing Xu. "Enhanced Intracellular SiRNA Delivery Using Bioreducible Lipid-Like Nanoparticles." Advanced Healthcare Materials, September 1, 2014. https://doi.org/10.1002/adhm.201400039.

Exploring the Effectiveness of Sodium Lauryl Ester Sulfate as a Decellularizing Solution for the Ovaries of Female, Prepubescent Leukemia Patients

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Abstract

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,[2] however the inactivity of the hypothalamic-pituitary-ovarian axis prevents oocyte and embryo cryopreservation in prepubertal leukemia patients.[8] These methods also hold a high risk for reintroduction of malignant cells upon retransplantation.[6] The use of bioengineered ovaries would allow normal function of the ovaries without the risk of reintroducing malignancy.[5] 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 2.0mm. The samples were then decellularized with 1% SLES for 48 hours at 18-20°C. They were 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.[1]

Introduction

Leukemia is a malignancy that affects the blood and bone marrow of the patient.[3] 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.[2] 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 .[7] 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.

Methods and Materials

In this study, ovarian tissue samples came from patients, aged 18-35 years old, undergoing sexual reassignment surgery. Upon harvest, the samples were rinsed with phosphate buffered saline (PBS) and then frozen and stored at -80°C. Before the decellularization process, the samples were bisected and the cortex was cut into strips of about 2.0 mm. They were then decellularized in a **1% SLES** for 48 hours at 18-20°C, on a magnetic stirrer at 100 RPM and then in DNase 1 and PBS for 24 hours at 36°C. PBS was used multiple times to effectively rinse the chemicals and remaining cells from the ovary. Tissue samples fixed in a 10% neutral buffered formalin were then stained using hematoxylin and eosin (H&E) and Hoechst, to analyze and confirm complete decellularization. The integrity of the extracellular matrix was evaluated using Heidenhain's AZAN. To assess cytotoxicity and compatibility, human Wharton's jelly mesenchymal stem cells were cultured at 5% CO_2 and 37°C. The ovaries were also tested in vivo on 8 week old rats. Primary ovary cells (POCs) from the rats were seeded in the scaffold and incubated to determine the viability of the cells.[1]

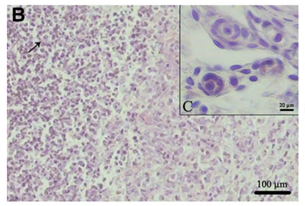


Figure 1: Viable ovarian cell populations on the POCs seeded scaffold.[1]

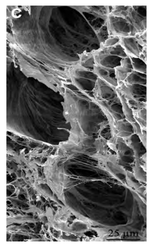


Figure 2: Decellularized ovarian samples with good preservation of integrity and structure.[1]

Results

Throughout the decellularization process, the human ovaries preserved their shape and did not present any deformities as a result of the SLES, as seen in figure 2. The H&E stain showed an absence of cellular material and a decrease in DNA content. Heidenhain's AZAN revealed the presence of Collagen 1, an important protein of the ovarian interstitial matrix, throughout the decellularized cortex and medullary areas. The ECM remained intact and unaffected, as well as the collagen. The MTT test, performed on the rats, revealed that the human Wharton's jelly mesenchymal stem cells were viable, could proliferate the scaffold, and penetrated into the matrix. In the POCs-seeded scaffolds, there was a high number of viable cell populations. Primordial follicle structures were also visible. Some oocytes surrounded by granulosa cells were visible.[1]

Applications to Biotechnology

As the survivorship of childhood leukemia increases, the consequences of cancer treatment on fertility have come into consideration. The decellularization process of ovarian tissue remains a crucial step into restoring the fertility of prepubescent female leukemia patients. The effectiveness of SLES demonstrated by the study would allow for the progression of the development of bioengineering ovaries. SLES offers a safer and more effective alternative to other detergents that are the subjects of other studies for the decellularization of other organs. The further development of this process would allow a safe fertility treatment option for female childhood leukemia survivors treated before puberty.

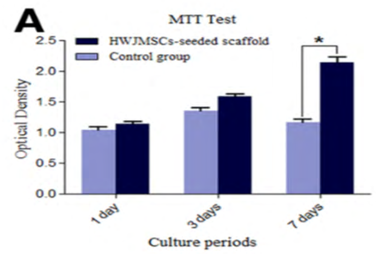


Figure 3: Cytocompatibility of the scaffolds was tested through comparisons of the HWJMSC cultures to the control group at 1, 3, 7 days.[1]

Rats	Grafts	Follicles				
		Primordial follicles	Primary follicles	Total surface area analyzed (μm^2)	Number of primordial follicles/ μm^2	Number of primary follicles/ μm^2
1	Right	5	7	1,012,500	4.9×10^{-6}	6.9×10^{-6}
	Left	7	9	984,236	7.1×10^{-6}	9.1×10^{-6}
2	Right	11	4	1,142,153	9.6×10^{-6}	3.5×10^{-6}
	Left	7	10	1,000,971	7×10^{-6}	1×10^{-5}
3	Right	14	6	924,048	1.5×10^{-5}	6.5×10^{-6}
	Left	9	6	1,011,689	8.9×10^{-6}	5.9×10^{-6}
4	Right	0	0	1,004,704	0	0
	Left	8	5	899,710	8.9×10^{-6}	5.6×10^{-6}
Mean ± SD		7.63 ± 4.14	5.88 ± 3.09	997,501.38 ± 72,258.24	$7.7 \times 10^{-6} \pm 4 \times 10^{-6}$	$5.9 \times 10^{-6} \pm 3 \times 10^{-6}$

Figure 4: The number of primordial and primary follicles in the POCs-DS grafts four weeks post transplantation.[1]

Discussion

The 1% SLES demonstrated the ability to effectively decellularized the ovarian samples, while maintaining the integrity and shape of the ovary and ECM. The preservation of the ECM remains essential to facilitating follicle growth, essential to the restoration of fertility. In addition to the decellularization ability of the SLES, the ovary remained able to facilitate normal and healthy growth of stem cells, as well as primordial follicles, oocytes and granulosa cells. The ovarian scaffolds treated with the 1% SLES were able to recreate the normal environment of a healthy ovary.[1] While similar decellularization process have been a focus of some studies, such as SDS, SLES is able to maintain the ECM, crucial to the ovary's viability.[4]

Acknowledgments

I would like to thank Dr. Ericka Senegar-Mitchell for all of the encouragement and guidance she has provided throughout the ROSA program. I would also like to thank all of the doctors and scientists who made ROSA possible, and took time to teach and mentor my sisters and I. I am also grateful for my ROSA sisters and mentor, Yasmin, for the support and positivity we offered each other.

References

1. Hassanpour, A., Talaei-Khozani, T., Kargar-Abarghoei, E., Razban, V., & Vojdani, Z. (2018). Decellularized human ovarian scaffold based on a sodium lauryl ester sulfate (Sles)-treated protocol, as a natural three-dimensional scaffold for construction of bioengineered ovaries. Stem Cell Research & Therapy, 9(1), 252. https://doi.org/10.1186/s13287-018-0971-5
2. Hunt, S., & Vollenhoven, B. (2019). Fertility preservation in women with cancer and afterward. Climacteric, 0(0), 1–5. https://doi.org/10.1080/13697137.2019.1607285
3. Juliusson, G., & Hough, R. (2016). Leukemia. In D. P. Stark & G. Vassal (Eds.), Progress in Tumor Research (Vol. 43, pp. 87–100). https://doi.org/10.1159/000447076
4. Kawasaki, T., Kirita, Y., Kami, D., Kitani, T., Ozaki, C., Itakura, Y., … Gojo, S. (2015). Novel detergent for whole organ tissue engineering: Organ Tissue Engineering for Various Organs. Journal of Biomedical Materials Research Part A, 103(10), 3364–3373. https://doi.org/10.1002/jbm.a.35474
5. Laronda, M. M., Rutz, A. L., Xiao, S., Whelan, K. A., Duncan, F. E., Roth, E. W., … Shah, R. N. (2017). A bioprosthetic ovary created using 3D printed microporous scaffolds restores ovarian function in sterilized mice. Nature Communications, 8, 15261. https://doi.org/10.1038/ncomms15261
6. Pors, S. E., Ramløse, M., Nikiforov, D., Lundsgaard, K., Cheng, J., Andersen, C. Y., & Kristensen, S. G. (2019.). Initial steps in reconstruction of the human ovary: Survival of pre-antral stage follicles in a decellularized human ovarian scaffold. Human Reproduction. https://doi.org/10.1093/humrep/dez077
7. Rosendahl, M., Andersen, M. T., Ralfkiær, E., Kjeldsen, L., Andersen, M. K., & Andersen, C. Y. (2010). Evidence of residual disease in cryopreserved ovarian cortex from female patients with leukemia. Fertility and Sterility, 94(6), 2186–2190. https://doi.org/10.1016/j.fertnstert.2009.11.032
8. Salama, M., Anazodo, A., & Woodruff, T. K. (2019). Preserving fertility in female patients with hematological malignancies: The key points. Expert Review of Hematology, 12(6), 375–377. https://doi.org/10.1080/17474086.2019.1613150

Effect of hMG on Oocyte Development During In Vitro Maturation

Cassidy Kirk · Madison High School

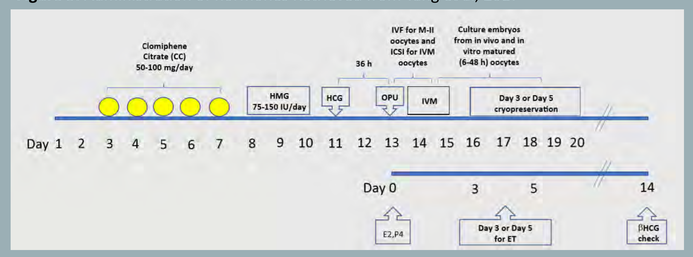
Abstract

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 give patients the best ability to protect the health of the mother and her child.

Figure 3: Administration of hormones Retrieved from Yang et al, 2017



Methods and Materials

In the "Endocrine" study, all patients with normal functional ovarian reserves (NFOR) were given a standard mixture of 8.1 mIU/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 of their patient with 75-150 IU/day of hMG for 3 days, varying based off of the size of follicles, 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. Singletons, 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

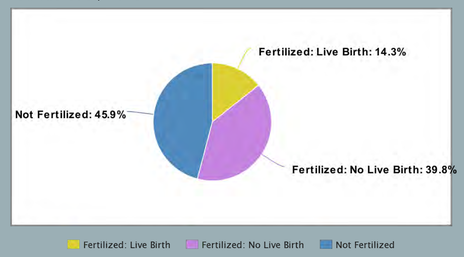
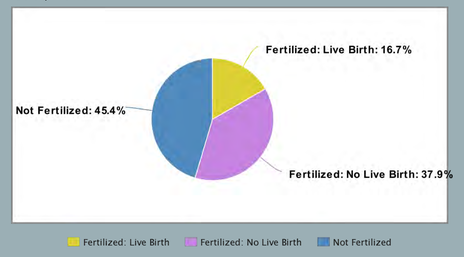


Figure 2: hCG Stimulated COC Results Adapted from Galvão et al, 2018



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, which 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 we hoped it would, this 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 like polycystic ovary syndrome.

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References

1. Forman, E., Anders, C., & Behera, M. (2008). A nationwide survey of oncologists regarding treatment-related infertility and fertility preservation in female cancer patients. *Fertility and Sterility, 90*. doi:10.1016/j.fertnstert.2008.07.1204

2. Galvão, A., Segers, I., Smitz, J., Tournaye, H., & Vos, M. D. (2018). In vitro maturation (IVM) of oocytes in patients with resistant ovary syndrome and in patients with repeated deficient oocyte maturation. *Journal of Assisted Reproduction and Genetics, 35*(12), 2161-2171. doi:10.1007/s10815-018-1317-z

3. Kedem, A., Hourvitz, A., Yung, Y., Shalev, L., Yerushalmi, G. M., Kanety, H., . . . Maman, E. (2013). Anti-Müllerian hormone (AMH) downregulation in late antral stages is impaired in PCOS patients. A study in normo-ovulatory and PCOS patients undergoingin vitromaturation (IVM) treatments. *Gynecological Endocrinology, 29*(7), 651-656. doi:10.3109/09513590.2013.798279

4. Lee, H., Barad, D. H., Kushnir, V. A., Shohat-Tal, A., Lazzaroni-Tealdi, E., Wu, Y., & Gleicher, N. (2015). Rescue in vitro maturation (IVM) of immature oocytes in stimulated cycles in women with low functional ovarian reserve (LFOR). *Endocrine, 52*(1), 165-171. doi:10.1007/s12020-015-0744-1

5. Yang, Z., M.D., & Chian, R., Ph.D. (2017). Development of in vitro maturation techniques for clinical applications. *Elsevier Inc.* doi:10.1016/j.fertnstert.2017.08.020

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.[4] 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 cell deaths, 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.[2] 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.[3]

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 doses of drugs in chemotherapy, particularly doxorubicin, due to its toxic side effects such as skin eruptions, dilated cardiomyopathy, 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.[4]

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

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 though 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 minimally 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 demonstrate 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.

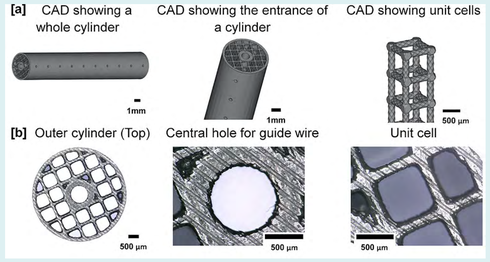

Figure 2. Design of the 3D printed porous cylinder using computer-aided design (CAD) and optic micrographs of its interior. [4]

The cylinders were printed with the material polyethylene glycol diacrylate (PEGDA). The surfaces of the cylinders were coated with a poly(*tert*-butylstyrene)-*b*-poly(ethylene-*co*-propylene)-*b*-poly(styrene-*co*-styrenesulfonate)-*b*-poly(ethylene-*co*-propylene)-*b*-poly(*tert*-butylstyrene) (PtBS–PEP–PSS–PEP–PtBS) block copolymer. After the 3D printed cylinders were fitted into silicone tubing, the copolymer solution was pumped through the cylinders for 10 minutes. Then, the cylinders were left to air dry. This left the cylinders with a thin coating of the copolymer on their surfaces. The coating thickness was mostly uniform, ranging from 30 to 60 µm. The coating material polystyrene sulfonate was selected because of its high capacity for doxorubicin binding. The PtBS and PEP blocks in the copolymer are responsible for holding the coating and cylinder together.

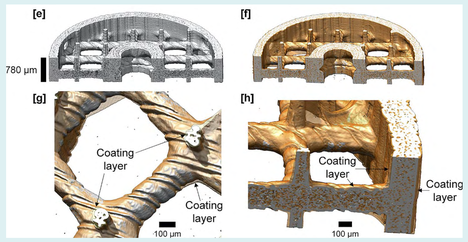

Figure 3. Magnified views of the uncoated (gray) and coated (orange) absorbers at different locations. [4]

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 common iliac vein over a span of 10 minutes. The dose represents a typical dose used in chemotherapy for intra-arterial treatment of hepatocellular carcinoma. Doxorubicin concentrations were monitored by three blood-sampling catheters placed at different locations. The three catheters include the pre-device catheter that is located between the drug injection and the absorber, the post-device catheter that is located closely downstream of the absorber, and a third catheter that was placed in the internal jugular vein (a peripheral location) to accurately measure systemic drug concentration.

Results

The experiment involved two groups: a control group wherein uncoated absorbers were deployed, and an experimental group with the coated absorbers. [3]

Control group: Uncoated absorbers were 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 concentration 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 centrifuged plasma 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.

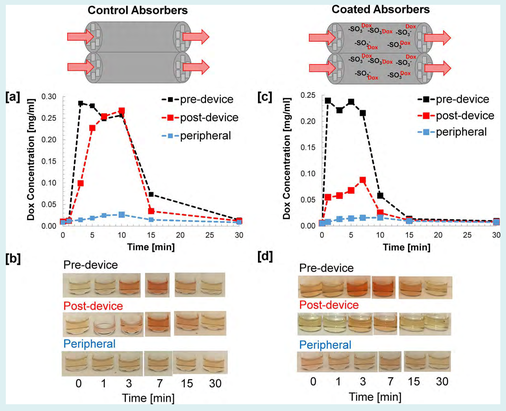

Figure 4. Results of the *in vivo* experiments:(left) two uncoated control absorbers and (right) two coated absorbers. Doxorubicin concentration in the blood as a function of time at the three different sampling locations for (a) uncoated absorbers and (c) coated absorbers. Photos of plasma from the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

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 any noticeable side effects. The performance of this 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 optimization to be done, 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.

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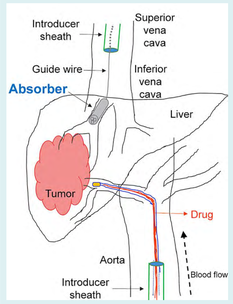
First and foremost, I would like to thank Dr. Ericka Senegar-Mitchell for her endless enthusiasm and infectious passion for science and for being the most inspiring big sister in science. A big thanks to Dr. Jeffery Chang, Dr. Irene Su, and all of our other guest lecturers for imparting their knowledge to us; Ms. Patricia Winter for organizing this amazing program; and Yasmin 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. [3]

Figure 5. Treatment of liver cancer by administering intra-arterial chemotherapy via the hepatic artery. [4]

References

1. Blumenfeld, C. M., Schulz, M. D., Aboian, M. S., Wilson, M. W., Moore, T., Hetts, S. W., & Grubbs, R. H. (2018). Drug capture materials based on genomic DNA-functionalized magnetic nanoparticles. *Nature Communications, 9*(1). doi:10.1038/s41467-018-05305-2
2. Chatterjee, K., Zhang, J., Honbo, N., & Karliner, J. S. (2010). Doxorubicin cardiomyopathy. *Cardiology*, *115*(2), 155–162. doi:10.1159/000265166
3. Chen, X. C., Oh, H. J., Yu, J. F., Yang, J. K., Petzetakis, N., Patel, A. S., . . . Balsara, N. P. (2016). Block Copolymer Membranes for Efficient Capture of a Chemotherapy Drug. *ACS Macro Letters,5*(8), 936-941. doi:10.1021/acsmacrolett.6b00459
4. Oh, H. J., Aboian, M. S., Yi, M., Maslyn, J. A., Loo, W. S., Jiang, X., … Balsara, N. P. (2019). 3D Printed Absorber for Capturing Chemotherapy Drugs before They Spread through the Body. *ACS central science*, *5*(3), 419–427. doi:10.1021/acscentsci.8b00700
5. Siegel, Rebecca L (2019). "Cancer Statistics, 2019". CA Cancer J Clin. 69 (1): 7–34. doi:10.3322/caac.21551. PMID. 30620402

Basic Fibroblast Growth Factor (bFGF) to Improve *In Vitro* Oocyte Maturation in a Tissue-Engineered 3D Culture System

Dhruti Pandya • Westview High School

Objective

Currently, ovarian tissue transplantation is the main fertility treatment option for prepubescent female cancer patients undergoing cytotoxic treatments, however, it risks reintroducing malignant cells.[5] *In Vitro* maturation is a cost-effective alternative without the risk of Ovarian Hyperstimulation Syndrome in PCOS and reintroducing malignant cells in cancer patients. Basic fibroblast growth factor (bFGF), a growth factor protein, is central to human cell proliferation, granulosa cell apoptosis inhibition, cumulus expansion, and meiotic resumption.[2,5] The goal of this meta-analysis is to investigate the role of bFGF in *in vitro* follicle growth and to explore the potential of utilizing bFGF and substances such as retinoic acid (RA) for a synergistic effect on follicular growth and oocyte maturation.[1,3]

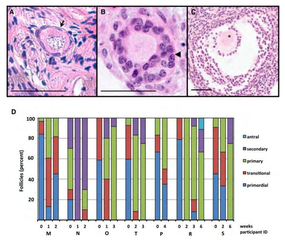


Figure 1: Alginate encapsulation and culture of human ovarian cortex supports folliculogenesis. Representative images of a) a primordial follicle, b) a secondary follicle, and c) an antral follicle within the cultured ovarian tissue are shown. The distribution of follicle classes at each culture time point is plotted for individual participants. *Retrieved from Laronda et al., 2014.*

Abstract

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.[5] 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 IVF.[3,4] 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.[2,3,5] In a study, ovarian tissue from 14 females aged 6-38 years was cultured in a cell medium supplemented with 0, 50, 100, or 300 ng/mL bFGF.[3] 60% of 107 follicles cultured with 300 ng/mL bFGF had increased E2 secretions and were developing after the fourth week compared to 4% of 181 follicles developing in the thawed control group.[3] In another study, 154 follicles were isolated from ovarian tissue from 11 women and encapsulated into 1% 3D alginate cultures with 0, 100, 200 or 300 ng/ml bFGF. After 8 days, the follicle diameters in the 200 ng/ml bFGF group were 133.3 ± 35.1 µm compared to 90.2 ± 29.8 µm in the 0 ng/ml bFGF group.[5] The survival rate of follicles in the group of 0 ng/ml bFGF was only 36.8% while the survival rate of follicles in the group of 100, 200 and 300 ng/ml bFGF increased to 73.8, 76.9 and 65.7%, thus indicating the advantageous effect of bFGF on *in vitro* oocyte maturation.[1,5] Further research is required on utilizing multiple growth factors for synergistic effects on follicle growth and developing sequential culture media to mature healthy human oocytes.[1]

Methods and Materials

Experiment 1: Ovarian tissue from 14 females aged 6-38 years were cut into 1-2mm slices and were frozen, thawed, fixed in Bouin's solution, and placed in Millicell CM inserts coated with an extracellular matrix gel. The slices were covered with a basic culture medium consisting of αMEM with 0.47 M ribonucleotides, 2 mM/L sodium pyruvate, 2 mM/L l-glutamine, 0.05% antibiotics, and 0.5 U/mL human recombinant FSH supplemented with a combination of 10% HSA, 1% insulin, transferrin, and selenium and 0, 50, 100, or 300 ng/mL bFGF. The follicle concentrations of E2 were measured by a double antibody radioimmunoassay kit and follicles were classified as primordial, primary, secondary, and antral after staining with hematoxylin and eosin.[3]

Experiment 2: 154 follicles obtained from 11 women were individually encapsulated into alginate (1% w/v) and randomly assigned to be cultured with 0, 100, 200 or 300 ng bFGF/ml for 8 days. Follicle viability was evaluated under confocal laser scanning microscope following Calcein-AM and Ethidium homodimer-I (Ca-AM/EthD-I) staining.[5]

Experiment 3: GV Oocytes obtained from 2-month-old NMRI mice were cultured in an α-MEM medium supplemented with 10% fetal bovine serum, 50 mg/l streptomycin, 60 mg/l penicillin and 10 ng/ ml epidermal 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, capacitated spermatozoa were added to in vitro matured oocytes which were assessed for cleavage to the two-cells stage.[1]

Results

Experiment 1: 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 bFGF in the E2 production of early follicles.[3]

Basic FGF Dose	Culture Period	Total No.	Primordial No.	Developing No.
Thawed Control		181	174	7 (4%)
100 ng/mL	Week 1	124	102 (46%)	122 (54%)
	Week 4	123	51 (41%)	72 (59%)
300 ng/mL	Week 1	153	70 (46%)	83 (54%)
	Week 4	107	43 (40%)	64 (60%)

Figure 2: Effects of varying doses bFGF on primordial follicle growth between 1 and 4 weeks of culture.[3]

Experiment 2: After 8 days in culture, all 154 follicles had increased in size. The diameter and survival rate of follicles in the group of 200 ng/ml bFGF was significantly higher than those in the group of 0 ng/ml bFGF (Figure 3).[5]

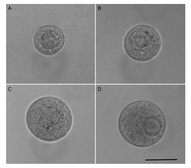


Figure 3: Characteristics of ovarian follicles growing *in vitro* with 200 ng/ml bFGF (A) Primary follicles (B) Secondary follicles (day 2) (C) Proliferated granulosa cells (day 4) (D) 3D structural integrity at Day 8. *Retrieved from Wang et al., (2014).*

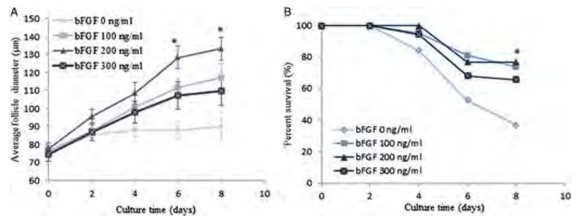


Figure 4: Larger diameters and increased survival rates of follicles grown with 200 ng/ml bFGF. *Retrieved from Wang et al., (2014).*

Experiment 3: As compared with the control group, the rate of maturation was significantly increased in the RA ($P<0.001$) and bFGF+RA ($P<0.02$) groups with 58 ± 10 and 57 ± 3.46, respectively. The bFGF+RA group had a higher rate 83 ± 1.52 (47.7%) of two-cells development, compared to the control 33 ± 1 (34%) ($P<0.001$) and bFGF rate 51 ± 1 (41%).[1]

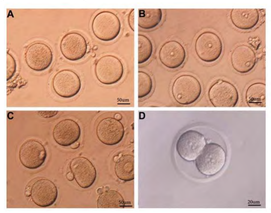


Figure 5: Oocytes in various stages of development. (A) Germinal vesicle breakdown (GVBD), (B) Germinal Vesicle, (C) Mature oocytes with polar bodies, and (D) Two-cells stage. *Retrieved from Abouzaripour et al., 2018.*

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 it 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 varying follicle stages in human follicular development.

Applications to Biotechnology

The relevance of this research lies in its potential to increase the survivability and maturation rate of human follicles *in vitro*. This research would not have been possible without developments in biotechnology including the 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.

Acknowledgements

I would like to thank Dr. Ericka Senegar-Mitchell for her mentorship and for inspiring me to be the best version of myself everyday. I would also like to thank Dr. Jeffrey Chang and Dr. Irene Su for giving me hands-on opportunities in labs and in the classroom, and Ms. Patricia Winter for helping organize this program. I'm grateful for an unforgettable summer with my ROSA sisters, and would like to thank my family for their continual support and my friends for brightening my days.

References

1. Abouzaripour, M., Fathi, F., Daneshi, E., Mortezaee, K., Rezaie, M. J., & Abdi, M. (2018). Combined Effect of Retinoic Acid and Basic Fibroblast Growth Factor on Maturation of Mouse Oocyte and Subsequent Fertilization and Development. International Journal of Fertility & Sterility, 12(1), 68-71. https://doi.org/10.22074/ijfs.2018.5293

2. Barros, R. G., Lima, P. F., Soares, A. C. S., Sanches, L., Price, C. A., & Buratini, J. (2019). Fibroblast growth factor 2 regulates cumulus differentiation under the control of the oocyte. Journal of Assisted Reproduction and Genetics, 36(5), 905-913. https://doi.org/10.1007/s10815-019-01436-7

3. Garor, R., Abir, R., Erman, A., Felz, C., Nitke, S., & Fisch, B. (2009). Effects of basic fibroblast growth factor on in vitro development of human ovarian primordial follicles. Fertility and Sterility, 91(5), 1967-1975. https://doi.org/10.1016/j.fertnstert.2008.04.075

4. Laronda, M. M., Duncan, F. E., Hornick, J. E., Xu, M., Pahnke, J. E., Whelan, K. A., … Woodruff, T. K. (2014). Alginate encapsulation supports the growth and differentiation of human primordial follicles within ovarian cortical tissue. Journal of Assisted Reproduction and Genetics, 31(8), 1013-1028. https://doi.org/10.1007/s10815-014-0252-x

5. Wang, T., Yan, L., Yan, J., Lu, C., Xia, X., Yin, T., … Qiao, J. (2014). Basic fibroblast growth factor promotes the development of human ovarian early follicles during growth in vitro. Human Reproduction, 29(3), 568-576. https://doi.org/10.1093/humrep/det465

Puberty Induced by Transplantation of Cryopreserved Ovarian Tissue

Elaine Yoon

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Objective

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.[1] 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, their 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. [5] 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 puberty. [1] With a way to induce puberty, young girls would be able to have their first period and ovulate as a result. In a case report by Ernst E., a 9 year old had a transplantation of cryopreserved ovarian tissue, and she regained ovarian function while secreting estradiol in a sufficient amount to induce puberty. [2] In another case recorded in 2003, a 10-year old girl had a transplantation of an autograft of cryopreserved ovarian tissue that was also used to induce puberty. [3] The similarities within each of these cases is the effect transplantation has on a woman's reproductive hormones. With more research, we can see how effective this process can be in a broad age range of prepubertal young girls. The transplantation of grafted or frozen ovarian tissue can induce puberty while also preserving fertility options later in the future. Finding ways to induce puberty should be the step taken prior to searching for fertility preservation options.

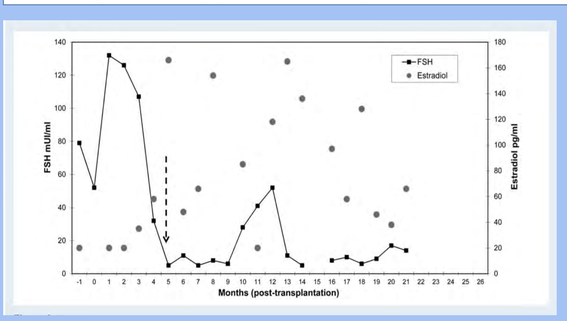

Figure 1. FSH/estradiol levels at and after transplantation with arrow indicating first menstruation. Adapted from Demeester et al.[1]

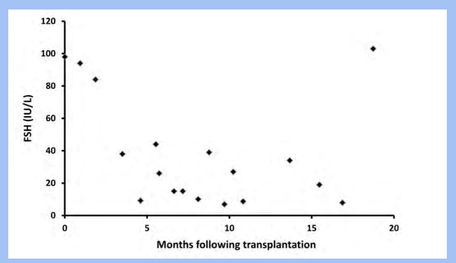

Figure 3. Levels of FSH following transplantation. Adapted from Ernst. E.[2]

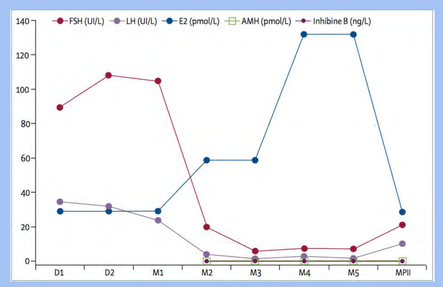

Figure 2. FSH, estradiol, inhibin B, and AMH levels at and after transplantation. Adapted from Poirot.[3]

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.[1]

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. [2]

In another case by Catherine Poirot, 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.[3]

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.[1]

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.[2]

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.[3]

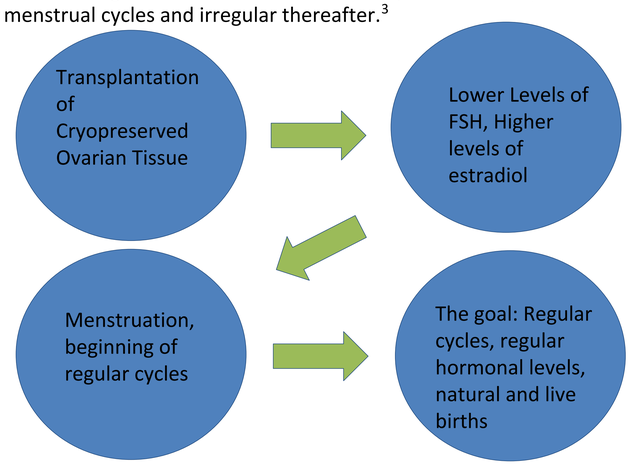

Figure 4. Adapted from discussion and analysis of research.

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 Inhibin 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. I also 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

1. Demeestere, I., Simon, P., Dedeken, L., Moffa, F., Tsépélidis, S., Brachet, C., . . . Ferster, A. (2015). Live birth after autograft of ovarian tissue cryopreserved during childhood: Figure 1. Human Reproduction, 30(9), 2107-2109. doi:10.1093/humrep/dev128

2. Ernst, E., Kjærsgaard, M., Birkebæk, N. H., Clausen, N., & Andersen, C. Y. (2013). Case report: Stimulation of puberty in a girl with chemo- and radiation therapy induced ovarian failure by transplantation of a small part of her frozen/thawed ovarian tissue. European Journal of Cancer, 49(4), 911-914. doi:10.1016/j.ejca.2012.09.028

3. Poirot, C., Abirached, F., Prades, M., Coussieu, C., Bernaudin, F., & Piver, P. (2012). Induction of puberty by autograft of cryopreserved ovarian tissue. The Lancet, 379(9815), 588. doi:10.1016/s0140-6736(11)61781-9

4. Raffel, N., Lotz, L., Hoffmann, I., Liebenthron, J., Söder, S., Beckmann, M., & Dittrich, R. (2017). Repetitive Maturation of Oocytes From Non-Stimulated Xenografted Ovarian Tissue From a Prepubertal Patient Indicating the Independence of Human Ovarian Tissue. Geburtshilfe Und Frauenheilkunde, 77(12), 1304-1311. doi:10.1055/s-0043-122601

5. Rivas Leonel, E., Lucci, C., & Amorim, C. (2019). Cryopreservation of Human Ovarian Tissue: A Review. Transfusion Medicine and Hemotherapy, 46(3), 173-181. doi:10.1159/000499054

DNA shed from endometrial and ovarian cancer can now be detected in pap smears

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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.

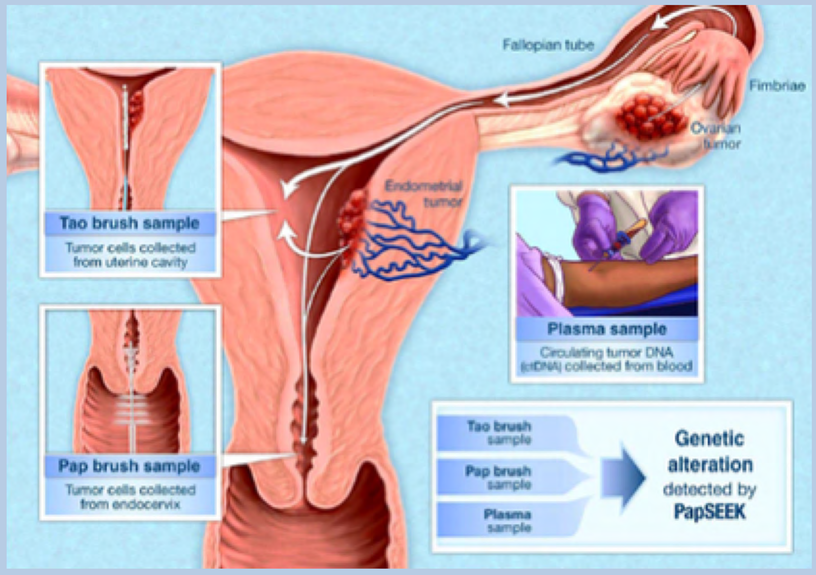

Fig. 1 visual from Johns Hopkins showing how the different tests are done ref. 2 (2018)

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 ctDNA (circulating DNA in the bloodstream) by focusing on 16 genes and using primers to look at 14 base pairs. In the study, there were two different types of brushes used to collect the samples the pap brush and the tao brush. The pap brush is the brush that is typically used in a pap smear to do a liquid biopsy on the cervix, so it could only collect the DNA from the tumors that were able to make it down to the cervix. The Tao brush was the other brush used in the study and used on 299 women, and it goes into the women's endometrial cavity to get a sample that was closer to the site of the tumors. The Tao brush was FDA approved and does not require anesthesia even though it goes through the cervical canal, but because of its small size and retractable covering it doesn't damage the cervix or cause the woman a lot of pain.

Abstract

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 shed and can be found on the cervix. The PapSEEK technique uses the sample that is taken from a pap brush 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.

Results

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

Fig 2. Chart made of the results of the study done at Johns Hopkins ref. 5 (2018)

When looking at the results both brushes were able to detect endometrial cancer very well, especially the Tao brush because the Tao brush can get a sample closer to the area of the cancer. For ovarian cancer, the Tao brush was noticeably better at detecting cancer, once again because it was able to get a sample closer to the source. The tao brush was also able to detect the highest percentage of early-stage ovarian cancers and that is thought to be because most ovarian cancers start in the fallopian tubes, then move to the ovaries. When looking at the results of looking for aneuploidy the numbers were low for both endometrial and ovarian cancer, so in the future that might not be the most helpful diagnostic tool. Finally when looking at the results of testing the plasma of the women with endometrial cancer the percentages go up substantially for women who either had mutations in their plasma or their pap brush sample, but testing plasma could be problematic in the future because cancerous ctDNA could come from anywhere in the body.

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.

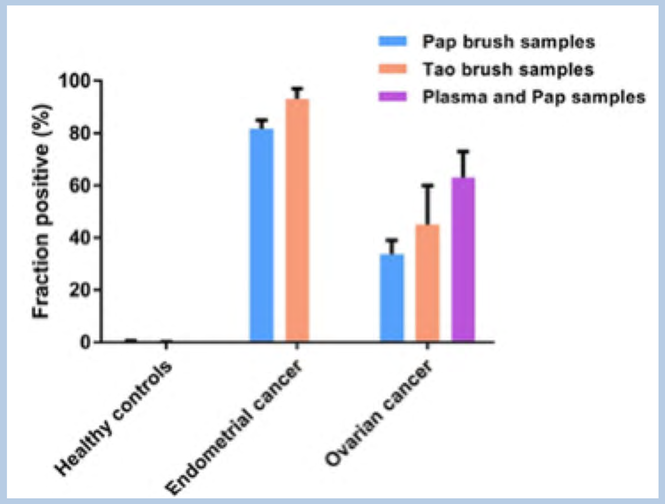

Fig. 3 bar graph showing results of testing the pap brush, the tao brush, and the plasma for ovarian cancer ref. 5 (2018)

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References

1. Bakkum-Gamez, J., & Dowdy, S. (2014, January 1). Retooling the Pap Smear for Ovarian and Endometrial Cancer Detection. Retrieved from http://clinchem.aaccjnls.org/content/60/1/22.long
2. Frum, L., & Mone, A. (2018, March 22). Pap Test Fluids Used In Gene-Based Screening Test for Two Gyn Cancers. Retrieved from https://www.hopkinsmedicine.org/news/newsroom/news-releases/pap-test-fluids-used-in-gene-based-screening-test-for-two-gyn-cancers
3. Kinde, I., Bettegowda, C., Wang, Y., Wu, J., Agrawal, N., Shih, I.-M., … Diaz, L. A. (2013, January 9). Evaluation of DNA from the Papanicolaou test to detect ovarian and endometrial cancers. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3757513/
4. Lowy, D., Nci, & Nci. (2018, May 2). PapSEEK Test for Endometrial and Ovarian Cancer. Retrieved from https://www.cancer.gov/news-events/cancer-currents-blog/2018/liquid-biopsy-screening-test-endometrial-ovarian
5. Wang, Y., Li, L., Douville, C., Cohen, J. D., Yen, T.-T., Kinde, I., … Papadopoulos, N. (2018, March 21). Evaluation of liquid from the Papanicolaou test and other liquid biopsies for the detection of endometrial and ovarian cancers. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6320220/

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

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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.[1] 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 68% 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.[7] RNF212 likely enhances DSB-induced oocyte apoptosis regulated by TAp63.[3,4,7] Another study analyzed the mechanism that TAp63-mediated 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.[3] 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.

Background

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.[6]
- Anticancer therapies like chemotherapy and radiation are effective in eradicating cancerous cells but also may harm reproductive organs and glands relevant to fertility, which can result in permanent infertility.

DNA Damage-Induced Oocyte Apoptosis

- Apoptosis, or programmed cell death, is a normal process and allows organisms to maintain cellular homeostasis by promoting cell turnover and removing cells that are unnecessary, not functional, or potentially dangerous.
- Oocytes that experience DNA damage typically undergo apoptosis in order to prevent the propagation of harmful mutations in the female germline. Among the various types of DNA damage, DSBs, or double-strand breaks, are one of the most harmful to cells.

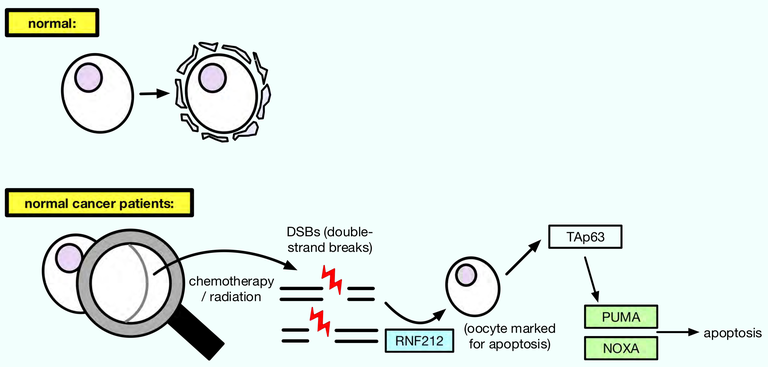

Figure 1. Schematic of expected pathways of oocyte development in healthy patients and cancer patients. Graphic created by E Kang, derived from findings of Kerr et al., 2012[3], and Qiao et al., 2018[7].

Materials and Methods Overview

RNF212

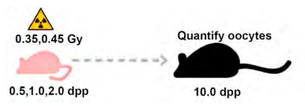

Figure 2. Visual representation of irradiation of WT and RNF212$^{-/-}$ mice. Retrieved from Qiao et al., 2018[7].

- 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 0.45 Gy.
- Oocytes were stained for γ-H2AX, a marker of double-strand DNA breaks, and quantified to assess DSBs and quantity of viable oocytes.

PUMA and NOXA

- PN5 PUMA$^{-/-}$, NOXA$^{-/-}$, PUMA$^{-/-}$NOXA$^{-/-}$ and, as controls, WT and Trp53$^{-/-}$ mice were exposed to to 0.45 or 4.5 Gy γ-irradiation.
- Oocytes were immunostained for γ-H2AX or phosphorylated ATM, an indicator of DNA damage and commencement of DNA repair.
- Female mice (WT, PUMA$^{-/-}$, and PUMA$^{-/-}$NOXA$^{-/-}$) were allowed to mature; when they reached 7 weeks of age, breeding trials were initiated with nonirradiated males.

Results

RNF212

- 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 γ-H2AX 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, **16% ± 3%** of primordial follicles in PUMA$^{-/-}$ mice and **52% ± 6%** in PUMA$^{-/-}$NOXA$^{-/-}$ mice survived; after 4.5 Gy γ-irradiation, **12% ± 1%** of primordial follicles in PUMA$^{-/-}$ mice and 94% ± 8% in PUMA$^{-/-}$NOXA$^{-/-}$ mice were protected from apoptosis **(Figure 6).**
- 13 out of 16 Puma−/− females and 9 out of 12 Puma−/−Noxa−/− females that were irradiated and mated produced **viable offspring**, whereas all irradiated WT females tested were **infertile**.

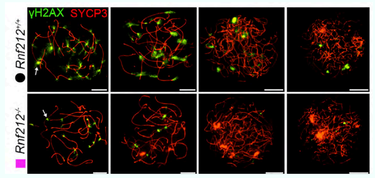

Figure 3 (left). 1-dpp oocyte nuclei immunostained for chromosome axis marker SYCP3 (red) and γ-H2AX (green). Retrieved from Qiao et al., 2018[7].

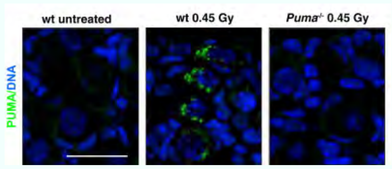

Figure 4 (right). PUMA antibody immunofluorescent staining in WT and PUMA$^{-/-}$ (negative control) primordial follicle oocytes, 6 hrs. after γ-irradiation. Retrieved from Kerr et al., 2012[3].

Applications to Biotechnology

This research is significant due to its potential on multiple fronts.

- 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 presented genes in human patients.
- Research in drug distribution is necessary in order to localize treatment and avoid the prevention of apoptosis in somatic cells, which may lead to uncontrolled cell proliferation that can progress into cancer.

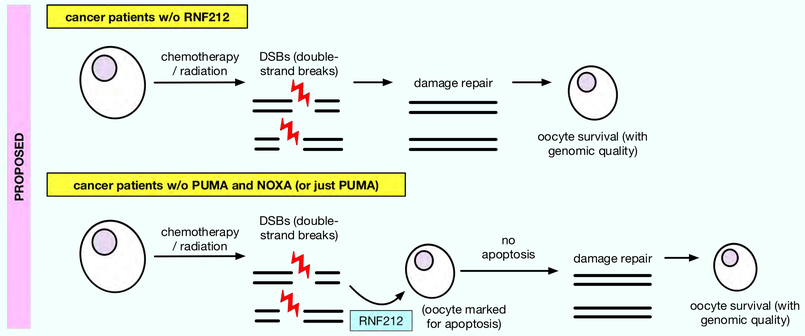

Figure 5. Schematic of oocyte development pathways resulting from the use of the proposed genes as drug targets. Graphic created by E Kang, derived from findings of Kerr et al., 2012[3], and Qiao et al., 2018[7].

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 knocking out 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 PUMA and NOXA during cancer treatment, fertility may be preserved by **preventing oocyte apoptosis, thereby allowing DNA repair and maintaining the ovarian reserve.**
- The **healthy offspring** resulting from irradiated PUMA$^{-/-}$ and PUMA$^{-/-}$NOXA$^{-/-}$ mice illustrate the potential for therapies that may target PUMA and NOXA in hopes of maintaining healthy oocytes so that a patient may be able to conceive.
- Manipulation of the expression of RNF212, PUMA, and NOXA has the potential to maintain oocyte quantity *and* genomic quality for cancer patients following intensive DNA damage-inducing treatments.

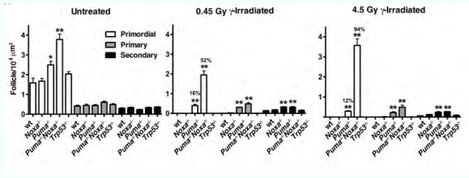

Figure 6 (left). Quantification of follicles from mice of the indicated genotypes either exposed to whole-body γ-irradiation (0.45 or 4.5 Gy) or untreated. Retrieved from Kerr et al., 2012[3].

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References

1. Carroll, J., & Marangos, P. (2013). The DNA damage response in mammalian oocytes. Frontiers in Genetics, 4. https://doi.org/10.3389/fgene.2013.00117
2. Collins, J. K., Lane, S. I. R., Merriman, J. A., & Jones, K. T. (2015). DNA damage induces a meiotic arrest in mouse oocytes mediated by the spindle assembly checkpoint. Nature Communications, 6(1). https://doi.org/10.1038/ncomms9553
3. Kerr, J. B., Hutt, K. J., Michalak, E. M., Cook, M., Vandenberg, C. J., Liew, S. H., … Strasser, A. (2012). DNA Damage-Induced Primordial Follicle Oocyte Apoptosis and Loss of Fertility Require TAp63-Mediated Induction of Puma and Noxa. Molecular Cell, 48(3), 343–352. https://doi.org/10.1016/j.molcel.2012.08.017
4. Kim, D.-A., & Suh, E.-K. (2014). Defying DNA Double-Strand Break-Induced Death during Prophase I Meiosis by Temporal TAp63 Phosphorylation Regulation in Developing Mouse Oocytes. Molecular and Cellular Biology, 34(8), 1460–1473. https://doi.org/10.1128/mcb.01223-13
5. Livera, G., Petre-Lazar, B., Guerquin, M.-J., Trautmann, E., Coffigny, H., & Habert, R. (2007). p63 null mutation protects mouse oocytes from radio-induced apoptosis. Reproduction, 135(1), 3–12. https://doi.org/10.1530/rep-07-0054
6. Siegel, R. L., Miller, K. D., & Jemal, A. (2019). Cancer statistics, 2019. CA: A Cancer Journal for Clinicians, 69(1), 7–34. https://doi.org/10.3322/caac.21551
7. Qiao, H., Rao, H. B. D. P., Yun, Y., Sandhu, S., Fong, J. H., Sapre, M., … Hunter, N. (2018). Impeding DNA Break Repair Enables Oocyte Quality Control. Molecular Cell, 72(2), 211–221.e3. https://doi.org/10.1016/j.molcel.2018.08.031

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, 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 inhibition of IGF-1 and negative side effects on other cell growth. Distinguishing disease-promoting from healthy macrophages is essential for the efficacy of this treatment.

Zhang et al. discovered miR-146b is prevalent in diseased macrophages through genotyping over 90 patient samples and determining cytokine production through subsequent ELISA. (6) Wu et al. found that curcumin, an antioxidant from the *Curcuma longa* herb, upregulates the expression of miR-146b while studying human glioblastoma. Sensitization from curcumin was quantified through rates of cell apoptosis and proliferation. (5)

A recommended area of study is to analyze whether the upregulation of miR-146b also causes the upregulation of IGF-1. This could form a drug that uses curcumin to make diseased macrophages more sensitive to linsitinib than healthy macrophages, thus specifically targeting the needed cells and reducing negative side effects. This treatment may have indications for women where treatment, such as GnRH-antagonist/estrogen depressant pills (3) and laparoscopic incision (1) is ineffective.

BACKGROUND

Endometriosis

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

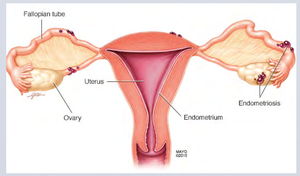

Figure 2. Endometriosis Illustration (Mayo Clinic)

Existing Treatments

- Elagolix
  - GnRH antagonist, decreases the production of estrogen
  - Forces the body into 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

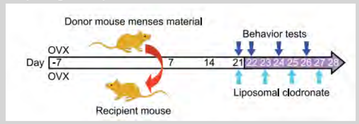

Figure 2. Treatment Timing (Forster, et al.) (2)

Expression Of IGF-1 In Diseased Macrophages

- Activated human peripheral blood monocyte-derived macrophages (MDMs) from healthy females with peritoneal fluids (PF) from diseased females to make *in vitro* endometriosis-associated macrophages (EAMs)

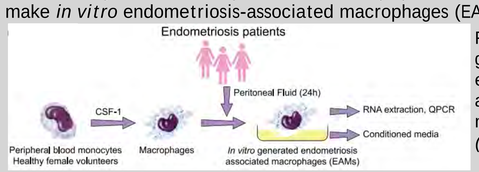

Figure 3. Process of generating endometriosis associated macrophages *in vitro* (Forster, et al.) (2)

- Pain from endometriosis was measured through mRNA expression of neurotrophic genes

Efficacy Of Linsitinib

- Linsitinib is an experimental drug
- Inhibited IGF-1 receptors through 40 mg/kg linsitinib
- Administered every 24 hours
- linisitnib is also an insulin receptor inhibitor

miR-146b in Diseased Macrophages

- Genotyping of miR-146b on peritoneal fluid from 74 diseased patients and 23 healthy controls
- Cytokine production from the macrophages were measured by enzyme-linked immunosorbent assay after overexpression and inhibition of miR-146b

Upregulating miR-146a

- Treated U-87 cells with different doses of curcumin for 48 hours
- RT-qPCR analysis afterwards
- (This paper worked with miR-146a, which is very similar to miR-146b)

RESULTS

Macrophages In Endometriosis

- Mice with endometriosis had increased levels of abdominal grooming and decreased levels of activity
- Mice with endometriosis and depleted macrophages had similar abdominal grooming to control mice but little increased activity
- Mice with endometriosis had medial prefrontal cortices that displayed increased mRNA expression of *Cox-2,* an inflammatory gene
- *Cox-2* was not expressed when macrophages were depleted

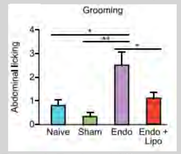

Figure 4. Treatment Affects on Abdominal Licking(Forster, et al.) (2)

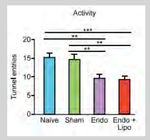

Figure 5. Treatment Affects on Activity (Forster, et al.) (2)

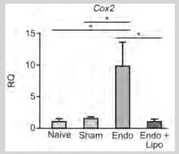

Figure 6. Treatment Affects on Cox2 (Forster, et al.) (2)

Expression Of IGF-1 In Diseased Macrophages

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

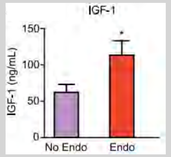

Figure 7. IGF-1 levels are higher in patients (Forster, et al.) (2)

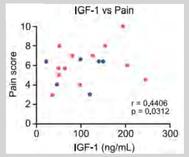

Figure 8. IGF-1 causes pain (Forster, et al.) (2)

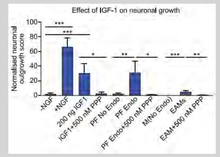

Figure 9. IGF-1 causes nerve growth in lesions (Forster, et al.) (2)

Efficacy Of Linsitinib

- Treatment caused decreased grooming in diseased mice but didn't affect activity levels
- Mechanical hyperalgesia in the abdomen and hindpaw returned to more normal levels

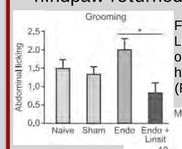

Figure 10. Linsitinib effects on grooming (Forster, et al.) (2)

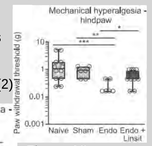

Figure 11. Linsitinib effects on abdominal hyperalgesia (Forster, et al.) (2)

Figure 12. Linsitinib effects on hindpaw hyperalgesia (Forster, et al.) (2)

miR-146b in Diseased Macrophages

- Expression of miR-146b was higher in endometriosis patients than control subjects

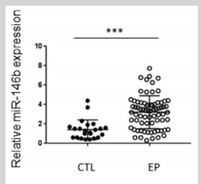

Figure 13. Endometriosis Expressing Patterns in Patients vs Control (Zhang, et al.) (6)

Upregulating miR-146a

- After being treated with curcumin for 48 hours, there was a significant increase in miR-146a
- The increase is dose-dependent

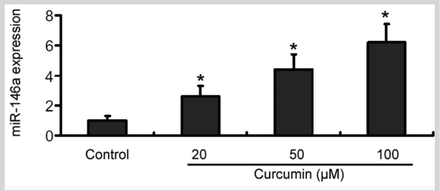

Figure 14. Curcumin upregulates miR-146a (Wu, et al.) (5)

DISCUSSION

- Pelvic pain is one of the main symptoms of endometriosis. Reducing hyperalgesia is a priority to improve patients' quality of life.
- To summarize, IGF-1 in macrophages causes pain —> linsitinib blocks IGF-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 IGF-1 and cause it to be more receptive to linsitinib?
- Integrating linsitinib and curcumin into a coherent drug that only targets IGF-1 in diseased macrophages

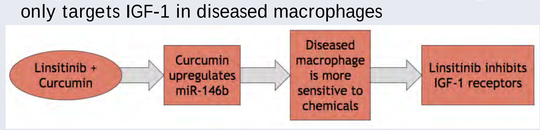

Figure 15. Future drug mechanism flow chart

BIBLIOGRAPHY

1. Byrne, D., Curnow, T., Smith, P., Cutner, A., Saridogan, E., & Clark, T. J. (2018). Laparoscopic excision of deep rectovaginal endometriosis in BSGE endometriosis centres: A multicentre prospective cohort study. BMJ Open,8(4). doi:10.1136/bmjopen-2017-018924
2. Forster, R., Sarginson, A., Velichkova, A., Hogg, C., Dorning, A., Horne, A. W., . . . Greaves, E. (2019). Macrophage-derived insulin-like growth factor-1 is a key neurotrophic and nerve-sensitizing factor in pain associated with endometriosis. The FASEB Journal,1-13. doi:10.1096/fj.201900797r
3. Lamb, Y. N. (2018). Elagolix: First Global Approval. Drugs, 78(14), 1501-1508. doi:10.1007/s40265-018-0977-4
4. New Endometriosis Treatment: What's the Latest? (n.d.). Retrieved from https://www.healthline.com/health/endometriosis/latest-endo-research
5. Wu, H., Liu, Q., Cai, T., Chen, Y., & Wang, Z. (2015). Induction of microRNA-146a is involved in curcumin-mediated enhancement of temozolomide cytotoxicity against human glioblastoma. Molecular Medicine Reports, 12(4), 5461-5466. doi:10.3892/mmr.2015.4087
6. Zhang, Z., Li, H., Zhao, Z., Gao, B., Meng, L., & Feng, X. (2019). MiR-146b level and variants is associated with endometriosis related macrophages phenotype and plays a pivotal role in the endometriotic pain symptom. Taiwanese Journal of Obstetrics and Gynecology,58(3), 401-408. doi: 10.1016/j.tjog.2018.12.003

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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) by the NADPH/dual specificity oxidases (NOX/DUOX) to activate mitogen activated protein kinase (MAPK) 1/3, but excessive ROS can distort GnRH signals and damage cellular structures[1]. 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 production[2,3], and diet induced obesity has been shown *in vivo* to inhibit ovulation in female mice[2]. Various plasma biomarkers of oxidative stress and serum FFA levels are increased with obesity, diabetes, and polycystic ovarian syndrome (PCOS)[3,4].

The sulfiredoxin-1 (SRXN1) reductase shows potential as a future drug target[1]. Normal and lentiviral *Srxn1* knockdown LβT2 cells were cultured with 10 nM GnRH for up to 6 hours or hourly. LβT2 cells were also treated with inhibitors of NOX/DUOX, MAPK 1/3, and ROS. RT-PCR, western blotting, wide field fluorescence imaging, and flow cytometry measured gene expression and cell ROS production. The results show that inhibition of NOX/DUOX, MAPK 1/3, and ROS significantly reduces *Srxn1* gene expression. Knockdown also significantly reduces baseline gonadotropin subunit mRNA and increases ROS production. These results show that SRXN1 is specifically targeted by GnRH signaling to reduce pituitary ROS and is essential for healthy hypothalamic-pituitary functions, showing potential as a drug target for treating obesity, diabetes, and PCOS related infertility.

Background

Sharma et al., 2013

Overview of the Estrous Cycle

In non-primate vertebrates, the estrous cycle is the main female reproductive cycle that is comparable to the human menstrual cycle. The first stage is proestrus, when the endometrium and ovarian follicles develop. The second stage is estrus, which is characterized by sexual receptivity. The third stage is metestrus, when sexual activity stops, the mature follicle is ovulated, and the corpus luteum forms. The last stage is diestrus, when the corpus luteum releases progesterone to be reabsorbed as nutrients. Although similar to the menstrual cycle, the estrous cycle follows different hormonal patterns in response to GnRH signaling[5].

Li et al., 2018

FFA Induces ROS Production

Women with obesity, diabetes, and PCOS are linked with reproductive dysfunction and have elevated levels of FFAs and oxidative stress. In FFA **β**-oxidation, fatty acid chains are converted to acyl-CoA and oxidized into shorter acyl-CoA chains and acetyl-CoA with the NAD+ and FAD coenzymes. This initiates the oxidative phosphorylation of ATP in the electron transport chain and generates ROS[6].

Kim et al., 2019

SRXN1 Reduces PRDX Antioxidant Enzymes

Peroxiredoxins (PRDX) are essential for reducing cellular ROS but can be inactivated by hyperoxidation. Peroxides are reduced at the active cysteine residue site (C_P) of PRDX, which oxidizes cysteine (C_P-SH) to sulfenic acid (C_P-SOH). C_P-SOH stabilizes by reacting with another cysteine residue (C_R). However, C_P-SOH can be hyperoxidized into sulfinic or sulfonic acid (C_P-SOOH/C_P-SOOOH) before resolution. Reduction by SRXN1 recycles hyperoxidized PRDX[7]. A main regulator of SRXN1 is the NRF2 transcription factor. KEAP1 inhibits NRF2, but ROS can dissociate KEAP1 by oxidizing cysteine residues[8].

Fig. 1: Illustration of the NRF2 signaling pathway. Adapted from Ahmed et al., 2016[8].

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) or a control low-fat diet (NC) for 24 weeks to induce obesity. Female mice were evaluated for estrous cycles after 6, 12, and 24 weeks on diet by vaginal lavage. Smears were classified as di/metetestrus, proestrus, and estrus based on cellular morphology. After 6, 12, and 24 weeks on the diet, blood was collected from the tail vein. Diestrus and estrus samples were taken at 12:00 PM, and proestrus samples were drawn between 5:45 and 6:15 PM. Mice were also stimulated with 1 μg/kg of GnRH 10 minutes, and blood was drawn before and after treatment. Plasma LH and FSH levels were measured by Luminex assay. To observe the effects of HFD 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.

To observe FFA β-oxidation effects on ROS production in GnRH signaling, mouse derived LβT2 cells were cultured with 500 μM ω-9 fatty acid oleate (OLA) or mOLA (which is resistant to metabolism) for 3 hours and stained with 5 μM CellROX Green oxidative stress reagent and 1 μM/mL Hoechst 33342 for wide-field fluorescent imaging and fluorescence intensity measurement. To observe FFA effects on LH and FSH levels, LβT2 cells were cultured with or without 300 μM OLA for 30 minutes prior to hourly pulses of 10 nM GnRH for 4 hours. LH and FSH levels were measured by immunoassay.

Control and S*rxn1* knockdown LβT2 cell lines were transduced with lentiviral shRNA particles for *Srxn1* (sh*Srxn1*) and pLKO.1-puro control transduction particles (shCTRL). Both cell lines were cultured with 10 nM GnRH for up to 6 hours or hourly. To test if *Srxn1* gene expression was induced by GnRH targeted signaling cascades or ROS, LβT2 cells were pretreated with NOX/DUOX (DPI), MAPK 1/3 (U0126 and PD098059), or ROS (NAC) inhibitors or not. To observe gonadotropin beta-subunit gene promoter activity, LβT2 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 reporter plasmid. After hormone treatments, RT-PCR measured of cDNA targets, and western blotting measured protein levels. To detect intracellular ROS accumulation, LβT2 cells were transfected with pLV-mCherry2-N1 plasmid and shCtrl plasmid or sh*Srxn1* pLKO.1 plasmid. Cells were stained with CellRox and 1 μM/mL Hoechst 33342 for 30 minutes and fixed in 4% formaldehyde for wide field fluorescent imaging and fluorescence intensity measurement. Flow cytometry detected cellular ROS production.

Results

Compared to NC mice, HFD mice have shorter proestrus, prolonged estrus, and fewer cycles, indicating abnormal estrous cycling. HFD mice lacked the proestrus LH and FSH surges and also had a significantly less corpus luteum (Fig. 2)[2]. However, HFD mice responded with a 3-fold LH increase to short term GnRH stimulation while FSH levels did not differ, indicating increased LH sensitivity in HFD mice.

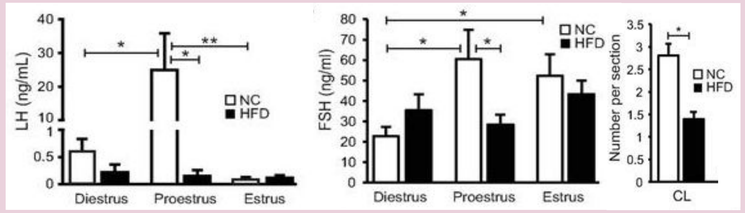

Fig. 2: Comparison of LH and FSH levels throughout estrous (n = 10/group) and the number of corpus luteum per 5 μm section (NC, n = 56; HFD, n = 77)[2].

Compared to controls, OLA treatment induced a ~2.5-fold change of ROS in LβT2 cells (Fig. 3)[3]. LH and FSH secretion were suppressed by OLA but not by mOLA, indicating that suppression resulted from β-oxidation. LH and FSH were most suppressed at the fourth hour, indicating decreased cell sensitivity over time.

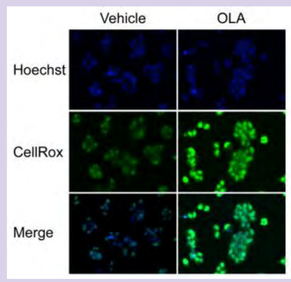

Fig. 3: A comparison of ROS induction by OLA in LβT2 and control cells (n ≥ 3)[3].

In *Srxn*1 knockdown cells, gonadotropin beta subunit mRNA (Fig. 4)[1] and reporter activity were significantly reduced. GnRH treatment of LβT2 cells for up to 6 hours resulted in a >50% increase in the oxidation of cytosolic PRDX 1/2 and mitochondrial PRDX 3 isoforms, indicating that GnRH induces PRDX hyperoxidation. *Srxn1* mRNA and SRXN1 protein levels increased ~25-fold in response to GnRH treatment over control values and peaked at 4 hours. Inhibition of NOX/DUOX activity, MAPK 1/3, and ROS significantly reduced SRXN1 increase in response to GnRH, indicating that SRXN1 is induced by GnRH and dependent on ROS. ROS production was greater in the sh*Srxn1* cells without GnRH and further amplified with treatment.

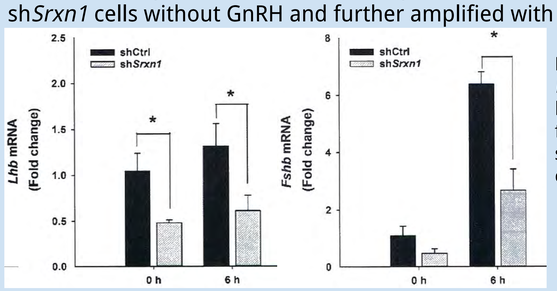

Fig. 4: Comparison of *Lhb* and *Fshb* mRNA levels at the first and fourth pulses between shCTRL and sh*Srxn1* cells (n = 3)[1].

Discussion

Women with obesity, diabetes, and PCOS have abnormal hypothalamic-pituitary activity, higher levels of FFAs, and heightened oxidative stress that sabotage fertility[3,4]. 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 FFA can distort GnRH signaling and impair the estrous cycle and ovulation. Li et al. shows that these phenomenons 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[2,3,6]. PRDX have been shown to reduce cellular ROS, but Kim et al. shows that GnRH signaling hyperoxidizes PRDX. SRXN1 reduces PRDX, and Kim et al. shows that SRXN1 is specifically targeted by GnRH signaling pathways and necessary for proper gonadotropin expression. However, SRXN1 requires hours of GnRH stimulation to reach high levels, 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

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References

1. Kim, T., Li, D., Terasaka, T., Nicholas, D. A., Knight, V. S., Yang, J. J., & Lawson, M. A. (2019). SRXN1 is Necessary for Resolution of GnRH-induced Oxidative Stress and Induction of Gonadotropin Gene Expression. *Endocrinology*. doi:10.1210/en.2019-00283
2. Sharma, S., Morinaga, H., Hwang, V., Fan, W., Fernandez, M. O., Varki, N., . . . Webster, N. J. (2013). Free Fatty Acids Induce *Lhb* mRNA but Suppress *Fshb* mRNA in Pituitary LβT2 Gonadotropes and Diet-Induced Obesity Reduces FSH Levels in Male Mice and Disrupts the Proestrous LH/FSH Surge in Female Mice. *Endocrinology, 154*(6), 2188-2199. doi:10.1210/en.2012-2218
3. Li, S., Mbong, E. F., John, D. T., Terasaka, T., Li, D., & Lawson, M. A. (2018). Induction of Stress Signaling In Vitro and Suppression of Gonadotropin Secretion by Free Fatty Acids in Female Mouse Gonadotropes. *Endocrinology, 159*(2), 1074-1087. doi:10.1210/en.2017-00638
4. Evans, J. L., Goldfine, I. D., Maddux, B. A., & Grodsky, G. M. (2002). Oxidative Stress and Stress-Activated Signaling Pathways: A Unifying Hypothesis of Type 2 Diabetes. *Endocrine Reviews,23*(5), 599-622. doi:10.1210/er.2001-0039
5. Allen, E. (1922). *The oestrous cycle in the mouse. American Journal of Anatomy, 30(3), 297–371.*doi:10.1002/aja.1000300303
6. Schulz, H., & Kunau, W. (1987). Beta-oxidation of unsaturated fatty acids: A revised pathway. *Trends in Biochemical Sciences,12*, 403-406. doi:10.1016/0968-0004(87)90196-4
7. Rhee, S. G. (2016). Overview on Peroxiredoxin. *Molecules and Cells,39*(1), 1-5. doi:10.14348/molcells.2016.2368
8. Ahmed, S. M. U., Luo, L., Namani, A., Wang, X. J., & Tang, X. (2017). Nrf2 signaling pathway: Pivotal roles in inflammation. *Biochimica Et Biophysica Acta (BBA) - Molecular Basis of Disease*, *1863*(2), 585–597. doi: 10.1016/j.bbadis.2016.11.005

Using the Post-implantation Amniotic Sac Embryoid (PASE) as an In Vitro Platform to Model Human Amniotic Sac Development

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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 when 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 organismal 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 a viable amniotic sac replica to solve technical and ethical challenges of harvesting and studying early human embryos.[2] One study showed the development of a biomimetic 3D culture system where hPSCs were placed as single cells onto different densities of Geltrex beds.[7] 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}.[7] Another study used immunofluorescence analysis to characterize cell fates. The results from staining showed that the columnar side of the asymmetric cyst is composed of epiblast-like cells that contain the pluripotency markers OCT4, NANOG and SOX2.[7] These same markers have been seen exclusively in the embryonic disc of post-implantation monkey embryos.[6] Immunofluorescence analysis of OCT4 also revealed that in day 5 PASE, there is EMT which is a phenotype associated with PS-initiation found in Carnegie stage 6 embryos.[7] 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.[3] Results showed if there is no Bmp2 or Smad5, there are defects in both amniotic and embryonic patterning.[7] Therefore, the PASE can approach the critical need for a viable in vitro platform to model and study key steps involved in human amniotic sac development.

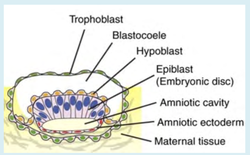


Figure 1. Drawing of implanting human embryo from Shao et al., 2017

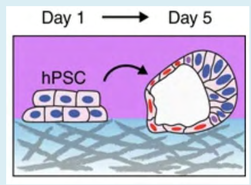


Figure 2. Drawing of 3D culture system and the formation of asymmetric cysts from hPSC from Shao et al., 2017

Methods

Experiment 1: *Analyzing Cell Plating Density in Geltrex 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 Geltrex. 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}).[7]

Experiment 2: *Immunofluorescence Analysis to Characterize Cell Fate*
The columnar side of the asymmetric cyst is composed of cells that retain 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 positive nuclei that were negative for pluripotency markers such as NANOG, OCT4 and SOX2. HOECHST was used for counterstaining cell nuclei.

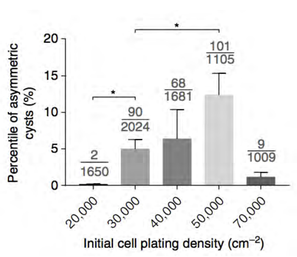
Experiment 3: *Immunofluorescence Analysis of OCT4 on PS-Initiation*
Through immunofluorescence analysis of the markers OCT4, NANOG and SOX2, it showed cells exit from pluripotency and suggested a non-neuroectodermal differentiation of these cells. These cells went through epithelial-to-mesenchymal transition, EMT, which is a phenotype associated with primitive streak (PS) initiation. Next, the transcription factor associated with PS development, BRACHYURY (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.[7]

Experiment 4: *BMP-SMAD in Embryonic Patterning*
A previous study of the PASE showed activated BMP-SMAD signaling during the development of hPSC ectoderm-like cyst. Furthermore, a study of embryogenesis in mice showed that BMP-SMAD signaling played an important role in amniotic tissue specification and morphogenesis, as loss of Bmp2 or Smad5 results in 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-SMAD in the PASE tissue.[7]

Results

Experiment 1: *Analyzing Cell Plating Density in Geltrex Beds*
Highest plating density produced exclusively columnar cyst on day 4 while lowest plating density produced full squamous amniotic ectoderm-like cyst on day 4. (Figure 3) The human amniotic sac consist of one side made up of squamous cells and one side columnar cells. A most accurate model of the human amniotic sac could only result from the intermediate cell plating density 30,000-50,000 cells cm^{-2} and not the other ranges. For PASE structures done in the rest of the experiments, the default range to create a similar model to the human amniotic sac was a density range of 30,000-35,000 cells cm^{-2}.[7] (Figure 4)

Figure 3. Graph that shows percentile of asymmetric cyst formed based on different plating densities from hPSC from Shao et al., 2017



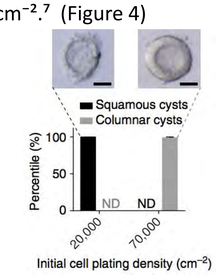


Figure 4. Graph that shows types of cyst formed on different plating densities from Shao et al., 2017

Experiment 2: *Immunofluorescence Analysis to Characterize Cell Fate*

The co-staining of OCT4 along with NANOG and SOX2 confirms the columnar side of the asymmetric cyst is composed of epiblast-like cells, which resembles the embryonic disc at one pole of the human sac. (Figure 5 and Figure 6) The markers OCT4, NANOG and SOX2 have been seen in co-staining used in embryonic disc of post-implantation cynomolgus monkey embryos. [7]

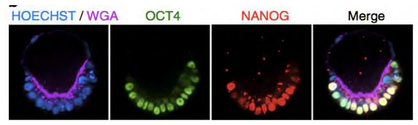


Figure 5. Immunofluorescence Analysis: staining of NANOG and OCT4 signify epiblast-like cells in PASE. Imaging from Shao et al., 2017

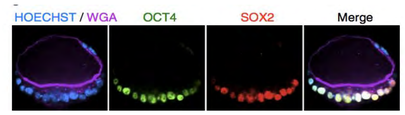


Figure 6. Immunofluorescence Analysis: staining of SOX2 and OCT4 signify epiblast-like cells in PASE. Imaging from Shao et al., 2017

Experiment 3: *Immunofluorescence Analysis of OCT4 on PS-Initiation*
In stage I of PASE, the model exhibits no cell dissemination and no prominent nuclear BRA in the embryonic disc. Stage II exhibits PASE that expresses nuclear BRA in the embryonic disc, but no cell emigration from the disc at all. Stage III displays PASE with cell emigration from a PS-like region flanked by the BRA, which is the columnar embryonic disc. (Figure 7) With the use of immunofluorescence analysis of OCT4, it is shown that there is a loss of pluripotency that turns into the formation of a PS-like region. The phenotype of BRA that coincides with the EMT process of OCT4 shows a resemblance to primitive streak development seen in post-implantation monkey embryos.[7]

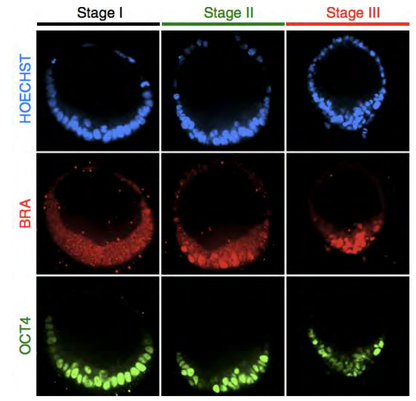


Figure 7. Images show both HOECHST and BRA staining seen in 3 stages of PASE. Imaging from Shao et al., 2017

Experiment 4: *BMP-SMAD in Embryonic Patterning*
The results show that SMAD1/5 emerges into the embryonic disc prior to CDX2, a marker for posterior/late PS. This is consistent with a finding that the activation of BMP-SMAD signaling precedes CDX2- mediated posterior PS specification. These results support the notion that balanced activation and inhibition of BMP-SMAD signaling may play an important role in maintaining the stable asymmetric amniotic ectoderm-epiblast tissue pattern during PASE development.[7]

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-SMAD signaling seen in embryonic discs of monkeys.[6] These experiments reveal 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 BMP-SMAD in PASE because this area seems unknown. Another goal is to examine the PASE model more than 5 days in culture, because all experiments were terminated in the PASE before effectively reaching 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 reproductive field. With the use of human pluripotent cells, there is potential that patients could receive transplants of tissue and cells without having to face problems like tissue rejection and tissue matching. The PASE model is just one example of hPSC potential to produce cells like that seen in the human. hPSCs could advance reproductive medicine and infertility solutions.

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References

1. Chen, D., Gell, J. J., Tao, Y., Sosa, E., & Clark, A. T. (2017). Modeling human infertility with pluripotent stem cells. Stem Cell Research, 21, 187–192. https://doi.org/10.1016/j.scr.2017.04.005
2. Jarvis, G. E. (2017). Early embryo mortality in natural human reproduction: What the data say. F1000Resear,52765. https://doi.org/10.12688/f1000research.8937.2
3. Nakamura, T., Okamoto, I., Sasaki, K., Yabuta, Y., Iwatani, C., Tsuchiya, H.,Saitou, M. (2016). A developmental coordinate of pluripotency among mice, monkeys and humans. Nature, 537, 57.
4. Nakamura, T., Yabuta, Y., Okamoto, I., Sasaki, K., Iwatani, C., Tsuchiya, H., & Saitou, M. (2017). Single-cell transcriptome of early embryos and cultured embryonic stem cells of cynomolgus monkeys. Scientific Data, 4(1), 170067. https://doi.org/10.1038/sdata.2017.67
5. Rivron, N., Pera, M., Rossant, J., Arias, A. M., Zernicka-Goetz, M., Fu, J., … Isasi, R. (2018). Debate ethics of embryo models from stem cells. Nature, 564(7735), 183–185. https://doi.org/10.1038/d41586-018-07663-9
6. Sasaki, K., Nakamura, T., Okamoto, I., Yabuta, Y., Iwatani, C., Tsuchiya, H., … Saitou, M. (2016). The Germ Cell Fate of Cynomolgus Monkeys Is Specified in the Nascent Amnion. Developmental Cell, 39(2), 169–185. https://doi.org/10.1016/j.devcel.2016.09.007
7. Shao, Y., Taniguchi, K., Townshend, R. F., Miki, T., Gumucio, D. L., & Fu, J. (2017). A pluripotent stem cell-based model for post-implantation human amniotic sac development. Nature Communications, 8(1), 208. https://doi.org/10.1038/s41467-017-00236-w
8. Taniguchi, K., Heemskerk, I., & Gumucio, D. L. (2019). Opening the black box: Stem cell–based modeling of human post-implantation development. The Journal of Cell Biology, 218(2), 410–421. https://doi.org/10.1083/jcb.201810084

Comparing the Efficacy of Progestin-Primed Ovarian Stimulation as an Alternative to GnRH Analogs

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Abstract

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, 0.34-38% of patients experienced premature LH surge.[3] This analysis aims to define the efficacy of Progestin-Primed Ovarian Stimulation[PPOS] compared to conventional GnRH analogs in blocking the LH surge, offering women better control of the oocyte production process and thus increasing the probability of completing in vitro fertilization. The studies in question completed randomized clinical trials 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 retrievals taking place after 12 days. Both groups were ultimately tracked for their mature oocytes to directly measure the effectiveness of progestins 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.[6] Martinez et al. carried out similar trials using desogestrel and found that fewer injections and a total lower cost produced similar oocyte yield and 52.0% vs. 58.6% birth rates.[4] 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 with varying 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.

Objective

GnRH analog therapies work to inhibit high LH and can allow oocyte maturation needed for IVF without losing oocytes to spontaneous ovulation. A novel substitute for conventional GnRH antagonists or agonists is PPOS. Progesterone interferes with the estradiol-induced positive feedback that makes LH within the activation stage, the earliest portion of the LH surge that involves the estradiol receptor neuronal system reading a stimulatory estradiol signal.[7]

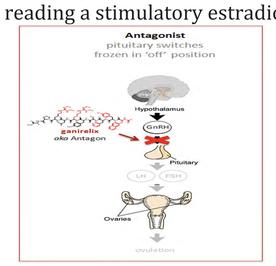

Figure 1. GnRH Antagonist Protocol. Adapted from *The Infertile Chemist.*[1]

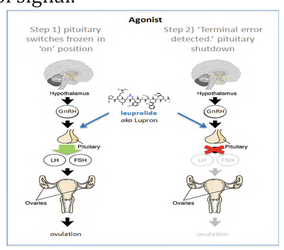

Figure 2. GnRH Agonist protocol. Adapted from *The Infertile Chemist.*[1]

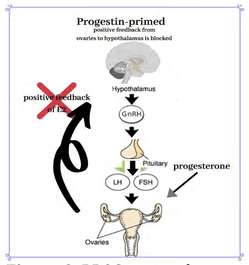

Figure 3. PPOS protocol. Adapted from Richter et al.[7]

Methods and Materials

Experiment 1 completed stimulation in patients under 41 years old with over 1.00 ng/ml of AMH in their first of second IVF/ICSI cycle. Patients with greater than 15 antral follicles or over 3.00ng/ml of AMH were given less HMG. 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 StatFlex v6.0.

Experiment 1 (Iwami et al. Trials)	GnRH Antagonist (Control)	Progestin-Primed (Experimental)
Time Intervals of Treatment	Daily when the leading follicle was 14mm	Daily from Day 2 to Day 12
Number of patients	126	125
Dosage of follicle stimulant	150-225 IU HCG	150-225 IU HCG
Dosage of Novel Therapy	0.25 mg subcutaneous injection	20mg dydrogesterone pill
Dosage of Final Trigger	GnRHa nasal spray/ additional 1000 IU hCG	GnRHa nasal spray/ additional 1000 IU hCG

Figure 4. This table demonstrates the amount of each therapy given over the 12 day stimulation period. Adapted from Iwami et al.[2]

Exp. 2 (Martinez et al. Trials)	GnRH Antagonist (Control)	Progestin-Primed (Experimental)
Time Intervals of Treatment	Daily from Day 2 to Day 12	Daily from Day 2 to Day 12
Number of patients	25	29
Dosage of follicle stimulant	100-150 IU corifollitropin alfa on Day 7/ Daily Gonadotropins Day 8-12	100-150 IU corifollitropin alfa on Day 7/ Daily Gonadotropins Day 8-12
Dosage of Novel Therapy	0.25 mg subcutaneous injection	Oral 75 mcg desogestrel
Dosage of Final Trigger	0.2mg GnRH agonist bolus	0.2mg GnRH agonist bolus

Figure 5. This table demonstrates the amount of each therapy given over the 12 day stimulation period. Adapted from Martinez et al.[4]

Experiment 3: Xiao et al. completed experimental trials with 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. For follicles of at least 14mm, 0.25mg of GnRH antagonist injections began to be administered daily. The study group received 150 to 225 IU/day hMG injections along with a daily oral 10mg MPA (medroxyprogesterone) pill after Day 3 of stimulation. In both experimental groups, the final trigger was induced by 0.2mg injection of decapeptyl and 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 a 16-G aspiration needle with ultrasound guidance. Statistical analysis was carried out using SPSS v16.0.

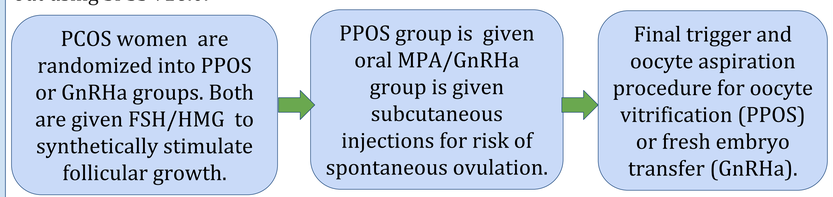

Figure 6. This diagram demonstrates the general procedure followed until IVF. Adapted from Xiao et al.[7]

Results

Experiment 1: There was no significant difference found within retrieved cluster-oocyte complexes (COCs), crates of oocyte maturation, embryo production, and rates of cycle cancellation. With a 79.6% mature oocyte rate for the PPOS protocol and 78.2% rate for the GnRH antagonist protocol, the two experimental groups are well within a considerable range of success. The study group had 1.85 mIU/ml of LH on trigger day while the control group had 2.74 mIU/ml, a significantly greater amount. Both groups exhibited no LH surge.

Characteristics	Dydrogesterone Group (Experimental)	GnRH antagonist (Control)	P Value
No. of MII oocytes	8.53 ± 5.39	8.71 ± 4.27	0.3499
Mature oocyte rate(%)	79.6 (1066/1339)	78.2 (1080/1381)	0.3684
Viable embryo rate(%)	65.5 (607/927)	68.1 (680/998)	0.2161

Figure 7. Outcomes of Ovarian Stimulation Protocol on Trigger Day. Adapted from Iwami et al.[2]

Experiment 2: The cycles using Desogestrel had significantly fewer subcutaneous injections than the GnRH antagonist cycles (10.34±2.83 vs. 5.03±2.12,p<.001). Because of these fewer injections, the average cost of each cycle under Desogestrel progestin was about 200 Euros less (1018.6 ± 191.0 vs. 813.8 ± 145.9, p <.001). Similar to Experiment 1, there were no significant differences in the number of oocytes retrieved from each woman between the PPOS and GnRH antagonist groups (14.09±6.86 vs. 15.91±8.09, p1⁄4.13). Regarding notable clinical effects, there were no cycle cancellations, no LH surges, and no rising exhibition of OHSS.

Experiment 3: In comparison to the control (GnRH antagonist) group, the study (progestin-primed) group had significantly lower levels of estradiol on the day of hCG trigger administration (3648±1838 *vs.* 4850±2538 pg/mL, *P*<0.001). Because of the lower estradiol levels, the progestin-primed study group resulted in lower rates of mature oocytes retrieved than the GnRH antagonist control group (14.24 ±8.25*vs.* 17.83±6.65, *P*=0.049). Despite this, the follow-through of preparing the oocytes maintained similar rates, including the number of 2PN fertilized oocytes, cleaved embryo, and 2PN fertilization.

Discussion

Despite there being no significant increase in oocytes per patient, the PPOS cycles kept similar levels in comparison to GnRH analogs. This means PPOS has potential as an alternative method for whom the conventional protocols are not ideal, without damaging general oocyte retrieval. PPOS currently can not be used for immediate embryo transfer and must be frozen or donated. Yet the oocyte donation process could become much cheaper because of oral LH inhibition over the more expensive injection method. The success of PPOS has implications for women already at risk of OHSS. Women suffering from PCOS are at high susceptibility for OHSS and may not want to undergo stimulation methods like the short protocol that raise this risk. The conventional protocols have also exhibited the incidence of follicular cysts as a direct result of the stimulation. Additionally, there is still minimal safety testing on GnRH-a in the body. PPOS is a relatively new consideration for researchers and much more work is necessary to determine the efficacy of the protocol on highly sensitive patients and how to prepare the body for fresh embryo transfer.

Relevant Applications to Biotechnology

This novel therapy has relevance to the bioengineering field of manufacturing methods of medicinal intake. Both short and long protocols involve relatively short DNA sequences that would be destroyed by enzymes if they began in the mouth which is why they are always injected subcutaneously. While GnRH antagonists and GnRH agonists currently can not be orally ingested without damage, future technologies could develop an oral method to make the stimulation process cheaper and simpler for oocyte maturation.

Acknowledgments

First and foremost, I would like to thank Dr. Senegar-Mitchell for promoting the educational pursuits and curiosity of all of the ROSA students. Thank you to Dr. Su, Dr. Chang, and Mrs. Winter who each invested heavy amounts of time into our classes, trips, and entire summer experience. Additionally, I would like to acknowledge the hard work of Yasmin Khajenoori for coming to our program every day and managing oversight across the past six weeks. I would also like to thank the entire ROSA guest faculty for spending their time to teach about their varying and groundbreaking work in both reproductive and cancer science. Finally, I am extremely grateful to find such a supportive group of fellow young, female scientists in my ROSA sisters.

References

1. Antagonistic. (2013, June 17). Retrieved from https://infertilechemist.com/tag/gnrh/
2.Iwami, N., Kawamata, M., Ozawa, N., Yamamoto, T., Watanabe, E., Moriwaka, O., & Kamiya, H. (2018). New trial of progestin-primed ovarian stimulation using dydrogesterone versus a typical GnRH antagonist regimen in assisted reproductive technology. *Archives of Gynecology and Obstetrics*, *298*(3), 663–671. https://doi.org/10.1007/s00404-018-4856-8
3.Marca, A. L., & Capuzzo, M. (2019). Use of progestins to inhibit spontaneous ovulation during ovarian stimulation: The beginning of a new era? *Reproductive BioMedicine Online, 39*(2), 321-331. doi:10.1016/j.rbmo.2019.03.212
4.Martínez, F., Rodriguez-Purata, J., Rodríguez, D. B., Clua, E., Rodriguez, I., & Coroleu, B. (2019). Desogestrel versus antagonist injections for LH suppression in oocyte donation cycles: a crossover study. *Gynecological Endocrinology*, *0*(0), 1–6. https://doi.org/10.1080/09513590.2019.1604661
5.Richter, T., Robinson, J., & Evans, N. (2002). Progesterone Blocks the Estradiol-Stimulated Luteinizing Hormone Surge by Disrupting Activation in Response to a Stimulatory Estradiol Signal in the Ewe. *Biology of Reproduction, 67*(1), 119-125. doi:10.1095/biolreprod67.1.119
6.Wang, Y., Kuang, Y., Chen, Q., & Cai, R. (2018). Gonadotropin-releasing hormone antagonist versus progestin for the prevention of premature luteinising hormone surges in poor responders undergoing in vitro fertilisation treatment: study protocol for a randomised controlled trial. *Trials*, *19*(1), 455. https://doi.org/10.1186/s13063-018-2850-x
7. Xiao, Z., Peng, J., Yang, J., & Xu, W. (2019). Flexible GnRH Antagonist Protocol versus Progestin-primed Ovarian Stimulation (PPOS) Protocol in Patients with Polycystic Ovary Syndrome: Comparison of Clinical Outcomes and Ovarian Response. *Current Medical Science*, *39*(3), 431–436. https://doi.org/10.1007/s11596-019-2055-x

Inhibition of MCT1 and MCT4 in Hypoxic Tumor Cells as a Non-Toxic Approach to Pancreatic Adenocarcinoma Cancer Recurrence

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Objective

The immunosuppressive and recurrent behavior of lactate from hypoxic tumor cells is significant in pancreatic adenocarcinoma (PDAC), which exhibits high relapse rates. [1,3,4] 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. [1,5] 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.

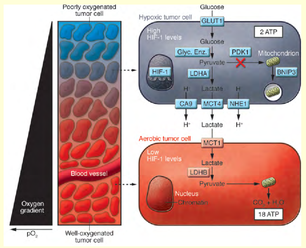

Figure 1: Mechanism of MCT lactate transport in hypoxic tumor cells
Retrieved from: Semeneza, G.L (1970) [7]

Abstract

Despite recent strides made in oncology, cancer recurrence has consistently eluded oncologists and researchers. No cancer better exemplifies this struggle than pancreatic adenocarcinoma (PDAC), which has a 5% 5-year survival rate and high risk of recurrence.[3,4] Hypoxic tumor cells, including those in PDAC, exhibit enhanced resistance to radiation and chemotherapy due to their metabolic reliance on lactate and are thought to be the primary perpetrators of cancer recurrence.[1,4] In the absence of lactate, hypoxic tumor cells suffer from glucose deprivation and become susceptible to radiation and chemotherapy.[2] Monocarboxylate transporters (MCTs) 1 and 4 control lactate uptake and transfer in hypoxic tumor cells and are not expressed in healthy pancreas cells.[1,5] Thus, targeting lactate metabolism in hypoxic pancreatic tumor cells via the inhibition of both MCT1 and MCT4 provides a promising non-toxic approach to PDAC cancer recurrence. In a recent study, 23 aminocarboxycoumarin derivatives were synthesized using palladium-catalyzed Buchwald–Hartwig type coupling reactions.[8] A primary assay was performed to identify compounds which selectively inhibited tumor cell proliferation (experimental cells derived from human cervix carcinoma cell line SiHa).[8] 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 a cell density of more than 50%.[8] In addition, the IC_{50} (compound concentration to reduce lactate uptake by 50%) and EC_{50} (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.[8] Though other substrates of MCT1 and MCT4 were identified, c19 is a promising candidate due to its excellent *in vitro* ADME and *in vivo* PK properties along with no anticoagulant side effects.[8] Its ability to minimize toxicity without compromising efficacy makes c19 a viable solution to pancreatic cancer recurrence.

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 MCTs, whereas the Wilson et al study and Voss et al study 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. [8] 23 aminocarboxycoumarin derivatives were synthesized using the Knoevenagel reaction and palladium-catalyzed Buchwald–Hartwig type coupling reactions. [8] For derivatives 17-23 in particular, various secondary amines replaced 3-bromophenol as the main building block. [8] A classical Vilsmeier-Haack reaction and cyclisation with Meldrum acid resulted in the final compounds. [8] A primary assay was performed to identify compounds which selectively inhibited tumor cell proliferation in lactate environments but displayed non-toxicity in glucose rich environments (experimental cells derived from human cervix carcinoma cell line SiHa). [8] 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. [8] The researchers then evaluated the in vitro ADME and in vivo PK profiles of c19 alongside performing a structure-activity relationship (SAR) analysis. [8] 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). [8]

Wilson et al study: rabbit erythrocytes were treated with pCMBS to determine whether pCMBS targets CD147. [10] The necessary data was "obtained by treatment of cells with the bifunctional organomercurial reagent fluorescein dimercury acetate that caused oligomerization of CD147." [10]

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 bioluminescence. [9] "The interaction of the full-length MCT1 or MCT4 with full-length Basigin provided the mechanism for complementation." [9]

Results

Drauoi 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. [8] Additionally, IC_{50} (compound concentration to reduce lactate uptake by 50%) and EC_{50} (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 (almost 3 log orders difference). [8] c19 exhibited excellent *in vitro* ADME and *in vivo* PK properties along with no anticoagulant side effects. [8]

Wilson et al study: Site-directed mutagenesis of CD147 suggested that the disulfide bridge in the C2 domain of CD147 is the target of pCMBS. [10] pCMBS strongly inhibited MCT1-CD147 as well as MCT4-CD147 interaction, preventing lactate transport into rabbit erythrocytes. [10]

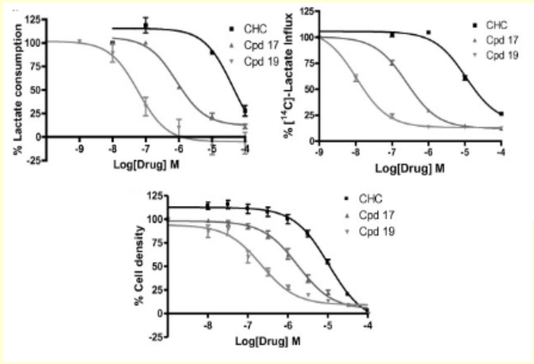

Figure 2: % lactate consumption, % [14C]-lactate influx, and % cell density of c17, c19, and CHC from enzymatic assays.
Adapted from: Draoui et al (2013) [8]

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. [9] 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". [9] 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." [9] ACF was found to have an IC_{50} of 5 μM. [9]

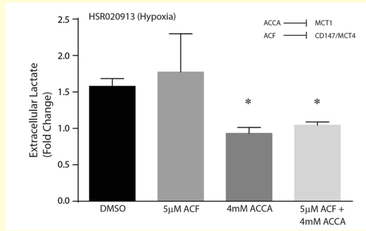

Figure 3: Examination of extracellular lactate levels in HSR-GBM1 treated in Hypoxia
Retrieved from: Voss et al (2017) [9]

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 IC_{50} than ACF, implying that hypoxia in PDAC would be far more receptive to c19 than ACF. [8,9] Though c19 was tested *in vivo* in mice via intraperitoneal injection, there is no data on c19 behavior specific to the pancreas, opening doors for further research. [8] pCMBS inhibits both MCT1 and MCT4 interaction with CD147, but there is no literature on its potential anticoagulant side effects or utility in cancer, specifically hypoxic tumor cells. [10] Finally, ACF demonstrates high efficacy when initially inhibiting MCT4 and CD147 interaction, but there is little published research on its anticoagulant side effects and no data on its behavior in the pancreas. [9] 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 success of ACF. [9] 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.

Taken all into consideration, c19 demonstrates the best potential as an non-toxic anticancer drug for two main reasons. First, c19 is a direct inhibitor of MCTs, which are suppressed in the healthy pancreas, so even though there is not enough data on effects of c19 in the pancreas, it can be deduced that c19 would likely not cause major adverse effects to normal pancreas function. [8] Furthermore, since c19 targets both MCT1 and MCT4 rather than only one of the MCTs, its chances of success significantly improve. [8] On the other hand, CD147 is highly expressed in 22.6% of normal pancreatic tissue, suggesting that using pCMBS or ACF to inhibit CD147 in PDAC could be toxic to healthy pancreatic cells (until future research can suggest otherwise). [9,10] Second, more research has to be done on pCMBS and ACF before their implications on normal pancreatic function and other parts of the body (such as anticoagulant effects) can be understood.

As oncologists continue their hunt for the best fertility-preserving treatments, targeting lactate metabolism via inhibition of MCT1 and MCT4 with c19 in the pancreas stands out. This method reduces risk of harm to reproductive organs by minimizing localized radiation exposure (by eliminating the immunosuppressive and radiation-resistant lactate-rich environment induced by hypoxia). [8] Also, given the submicromolar IC_{50} and EC_{50} values of c19, there is hope for a minimally-invasive, highly-localized, low-dose drug therapy for PDAC. [8] Overall, c19 has a plethora of benefits and presents itself as a promising solution to PDAC proliferation and recurrence.

Applications to Biotechnology

Using the latest nanotechnologies, c19 can be encased in nanoparticles and injected locally when being administered to patients. Polymer-drug nanoparticle conjugates offer improved water solubility and stability, longer circulation life, metabolic stability, site-specific targeting, and overall enhanced tumor penetration. [6] Prior research has also demonstrated the ability of nanoparticles to improve performance of cytotoxic agents in tumors. [6] The potential for c19 to be delivered via nanoparticle-encased drug should be considered.

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References

1.Halestrap, A. P., & Wilson, M. C. (2011, December 09). The monocarboxylate transporter family-Role and regulation - Halestrap - 2012 - IUBMB Life - Wiley Online Library. Retrieved from https://iubmb.onlinelibrary.wiley.com/doi/full/10.1002/iub.572
2.Lactic Acid Found To Fuel Tumors. (2008, November 23). Retrieved from https://www.sciencedaily.com/releases/2008/11/081120171325.htm
3.Long, J., Zhang, Y., Yu, X., Yang, J., LeBrun, D. G., Chen, C., . . . Li, M. (2011, July). Overcoming drug resistance in pancreatic cancer. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3111812/
4.Manzur, A., Oluwasanmi, A., Moss, D., Curtis, A., & Hoskins, C. (2017, September 25). Nanotechnologies in Pancreatic Cancer Therapy. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5750645/
5.Metran-Nascente, C., Yeung, I., Vines2, D. C., Green2, D., Milosevic2, M., & Jaffray2, D. (2016, March 01). Cristiane Metran-Nascente. Retrieved from http://jnm.snmjournals.org/content/57/3/361.full
6.Polymer-Drug Conjugates. (n.d.). Retrieved from https://www.sciencedirect.com/topics/medicine-and-dentistry/polymer-drug-conjugates
7.Semenza, G. L. (1970, January 01). [PDF] Tumor metabolism: Cancer cells give and take lactate. - Semantic Scholar. Retrieved from https://www.semanticscholar.org/paper/Tumor-metabolism:-cancer-cells-give-and-take-Semenza/324e3491a5745c203ec1d0ccfa84fa91c7a6c545
8.Synthesis and pharmacological evaluation of carboxycoumarins as a new antitumor treatment targeting lactate transport in cancer cells. (2013, September 13). Retrieved from https://www.sciencedirect.com/science/article/pii/S0968089613007815?via=ihub
9.Voss, D. M., Spina, R., Carter, D. L., Lim, K. S., Jeffery, C. J., & Bar, E. E. (2017, June 27). Disruption of the monocarboxylate transporter-4-basigin interaction inhibits the hypoxic response, proliferation, and tumor progression. Retrieved from https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5487345/#MOESM1
10.Wilson, M. C., Meredith, D., Fox, J. E., Manoharan, C., Davies, A. J., & Halestrap, A. P. (2005, July 22). Basigin (CD147) Is the Target for Organomercurial Inhibition of Monocarboxylate Transporter Isoforms 1 and 4. Retrieved from http://www.jbc.org/content/280/29/27213.long