EGFR-mutations Identified from Blood-Derived Circulating Tumor DNA in Patients with Advanced Lung Adenocarcinoma

Ruchi Agashe  
Canyon Crest Academy

**Methods and Materials cont.**

In the 88 tested patients, 27.3% (24 patients) were identified to have EGFR alterations. Patient data was analyzed in smokers compared to nonsmokers. Characterized EGFR mutations were more frequent in non-smokers (13% (13/95)) than in smokers (16% (8/50)). 34 patients (36%) with EGFR alterations also had a multigene panel NGS testing result. The median time interval between the blood draw and the tissue biopsy was 0.8 months. This timepoint was used as a cut point for comparing between tissue and ctDNA results. The overall concordance rate for EGFR alterations was 76.5%. When tissue testing of non-NSCLC types were included, the concordance rate was 75% compared to the median tissue biopsy time of 0.8 months, the concordance rate was 82.2%. When the time interval was greater than 0.8 months, the concordance rate was 64.7%. 26 patients (with ctDNA alterations) had both ctDNA and a common tissue molecular test. For these patients, the median time interval between the tissue biopsy to blood draw was 1 month. This analysis excluded patients with no ctDNA detected in their plasma. The overall concordance rate for tissue and ctDNA results is 82.6% for patients with ctDNA alterations. All 25 EGFR ctDNA alterations carrying EGFR mutations had negative results. It was possible for up to ctDNA alterations to detect EGFR alterations in ctDNA and were only positive for EGFR alterations in thectDNA. Of the 88 patients, 84% had an alteration in their ctDNA results that could be matched with a therapy. 80% of these (18 patients) received a targeted therapy directed to EGFR. Targets include activating EGFR mutations in exon 19, 85% in exon 21, 90% in exon 18 (for patients who have no EGFR alterations). ctDNA alterations, and 100% in patients whose time interval between the blood draw and tissue biopsy was less than 1 month. Of the 88, 46% of patients were matched to a therapy, 83% received matched therapy to EGFR mutations, and 73% achieved stable disease. In conclusion, liquid biopsy via ctDNA provides an affordable and effective way to detect resistance-generating mutations that can revolutionize cancer detection methods and improve matched therapies.

**Materials and Methods**

In the study, 88 consecutively tested patients with lung adenocarcinoma at UC San Diego Moores Cancer Center were reviewed. ctDNA was analyzed from the blood of 88 patients from August 2014 until October 2015. Digital sequencing of ctDNA in all of the patients was performed by the Guardant Health. 20 ml of heparinized plasma tubes were drawn from each patient. A total of 5 and 10 ml of plasma were extracted from the patients who had tissue and plasma samples. Sequencing libraries were prepared with custom bar-code molecular tagging. The capture is followed by NGS of critical exons in 70-80% of all tumors on four major types of genome alterations: point mutations, indels, fusions, and copy number amplifications. To remove false positives, bioinformatics is used to match complementary strands of each of the DNA fragments that are barcoded. The mutations are measured using the variant allele fraction (VAF), which is the number of mutated DNA molecules divided by the total number of DNA fragments at that allele.

For the detection of EGFR, a 45-mer panel was used to identify potential tumor-related alterations in 54 cancer-related genes (such as copy-number amplifications in EGFR). For 47 patients, a 50 gene panel was used to measure all alterations. For the patients who had ctDNA detected in their plasma, CDCA performed targeted therapy. A potential targeted gene alteration was characterized up to if there was any concordance between the ctDNA and tissue samples. A potentially actionable alteration is an alteration that has been identified in a clinical study to correlate with a specific outcome. The EGFR alterations targeted an EGFR inhibitor. For therapeutic efficacy the following were measured: rate of stable disease (SD) or partial response (PR), progression free survival (PFS), and overall survival (OS). SD, PR, and CR were determined by the treating physician. PFS was defined as the time from the beginning of the therapy to death or the last follow-up (for patients who were alive). The date of censoring was set as December 26, 2015. For statistical analysis, 2-tailed t-tests were used to compare categorical variables, nonparametric Mann-Whitney t-tests were used to compare two groups with a continuous variable, binomial logistic regressions were performed for categorical outcomes, and linear regressions were performed for continuous variables, and the Kaplan–Meier method was used to analyze PFS and OS. 

**Results**

**Discussion**

The order of most frequent alterations detected in the liquid biopsy showed similar rates to the Cancer Genome Atlas (TCGA). EGFR made up 27.3% of alterations in the experiment compared to 17% in the TCGA. Reasons for this might include different selection criteria for the liquid and solid biopsy, but the minor differences in survival may be related to the liquid biopsy data. Additionally, resistance mutations such as EGFR T790M are likely to be evaluated in the TCGA. Other reasons include differences in tissue and liquid biopsy data, where in the tumor, the patient is undergoing treatment. An analysis on the positive cases indicated that both tests and independently detect alterations not found in other tests, showing the clinical value of these technologies. An earlier research study identified between 86 and 91% of actionable mutations in the comparison of tissue and plasma results should be sampled during and before treatment to avoid ctDNA suppression. Reasons that may be possible in tissue but not ctDNA include decreased ctDNA shedding after treatment and not all tumors shed dna. ctDNA results may be positive when tissue DNA does not reflect the genetic footprint of multiple lesions. Additionally, gene mutations post-treatment may not be found and ctDNA is useful for matched therapies. When testing for actionable EGFR mutations, the initial detection of EGFR alterations in patients with advanced lung adenocarcinoma and monitoring during treatment is essential for detecting resistance mutations. Limitations to the study include: small sample size, patients didn't have concurrent tissue and ctDNA biopsies, range of intervals between ctDNA tests and tissue NGS, existence of patients without detectable ctDNA, and response assessment was performed by different physicians. In summary, the study reveals that ctDNA can be used as a liquid biopsy. The concordance rate of EGFR mutations confirms that a shortened time interval between blood collection and tissue NGS is preferable. Patients receiving cognate therapies had a higher rate of stable disease. Overall, multiple patients can benefit from ctDNA testing, including those with unclear tissue biopsy results, exhausted tissue from histopathology/immunohistochemistry testing, and no detectable actionable mutations. For patients with no clinical results from ctDNA testing, targeted therapy may be warranted. The advantage of ctDNA is the ability to detect the driving molecular alteration responsible for resistance in the bloodstream. Until then common use, ctDNA tests can be used to guide biopsy findings and search for possible genomic alterations after a differential diagnosis is reached.
Objective
The purpose of this poster is to examine the correlation between IVF/ICSI singletons and twins and detrimental outcomes in their future development. It will also discuss the impact of twinning on children conceived with and without assisted reproduction technologies. Twins born through IVF are particularly impacted physically and neurologically in this situation.

Abstract
Record numbers of women are turning to IVF with the hope of giving birth to a child despite infertility. While most IVF centers perform single embryo transfer, some will implant multiple embryos if a woman chooses to have twins or wants to have a higher rate of successful implantation. There is a clear difference in health outcomes between those infants conceived without assisted reproduction and those through IVF. A study done by the Groningen ART Cohort compared outcomes between three groups: children born through conventional IVF–ICSI vs. modified natural cycle IVF vs. natural conception. They studied 26 twin infants and 63 singletons, comparing rates of attrition in development after four years. A secondary study done in Denmark compared morbidity between 472 IVF/ICSI twins, 1132 non-IVF/ICSI twins and 634 IVF/ICSI singletons. The Groningen study demonstrated that 4-year-old IVF twins have a significantly lower total IQ, a lower body weight and a smaller height than 4-year-old IVF singletons. Supporting the primary findings, the Denmark study indicates that physical health of IVF/ICSI twins is comparable with that of non-IVF/ICSI twins. However, physical health of IVF/ICSI twins is poorer and the negative implications for the families are stronger compared with IVF/ICSI singletons. This supports that twins born through IVF have significantly decreased abilities in comparison to their peers. They have higher rates of anencephaly and preterm birth, as well as a significantly increased chance of respiratory complications, sepsis, and jaundice in later life. The information gathered in these studies raises concerns about the birth of multiple embryos, and indicate that single embryo transfer may be a better option.

Methods and Materials
In the Groningen ART Cohort study, the group followed up with offspring born through and without IVF to assess their health and development. Initially, they selected both sub fertile and fertile couples. From these, a total of 89 neonates born as a result of ovarian stimulation and IVF–ICSI, 26 twin infants and 63 singletons. The follow-up examination at the age of 4 years was carried out by trained researchers who were blinded to the mode of conception of the children. It consisted of the assessment of neurological and cognitive development and the evaluation of anthropometrics and blood pressure.

For neurological development, the HEMPS assessment, an age-specific neurodevelopmental examination, assessed neurological function in five domains: fine motor function, gross motor function, muscle tone and posture, reflexes and visual motor function. To evaluate cognitive development, the Kaufman Assessment Battery for Children was used to test IQ. The assessment of anthropometrics, or physical development, consisted of the measurement of height, weight, triceps skinfold and subscapular skinfold.

Results
According to the study, twins born through IVF/ICSI have increased implications in relation to neurological, and physical development. (See Figure 2 and 3). Twins had a lower birthweight (P < 0.001) and shorter gestational age at birth. Furthermore, IVF twins were more often delivered by Caesarean section and born preterm compared to singletons and non-IVF twins. This is supported by their higher admittance rate into the NICU (see Figure 1) and chance of morbidity. Post birth, it was found that the rate of attrition at the age of 4 years was 11% in the Groningen ART cohort study: 7% in singletons and 19% in twins. They had considerable differences between their means in the assessed categories of the study. For IQ, this was −5.4[−9.7 ‑ −1.0]. The difference between weight was −1.7[−2.7 ‑ −0.6], and height was −2.9[−5.0 ‑ −0.8]. Lastly, for blood pressure the mean difference was 0.5[0.0 ‑ 0.5]. These results align with the Danish study, which showed that compared with IVF/ICSI singletons, more IVF/ICSI twins had surgical interventions (P = 0.03), special needs (P = 0.02), and poorer speech development (P < 0.01).


Figure 2: Comparison of IVF singletons and twins with weight, standing height, standing blood pressure, and subscapular skinfold.

Figure 3: The learning IQ, knowledge IQ, total IQ, and percent of normal neurological outcome between IVF/ICSI singletons and twins.

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Conclusion
Neonatal outcomes are improved for IVF singletons compared with IVF twin births. The overall health of IVF/ICSI twins is poorer and the implications for the families stronger compared with IVF/ICSI singletons. They face more issues immediately, as well as in later life. Though the physical health of IVF/ICSI twins is comparable with that of non-IVF/ICSI twins, there still are some smaller differences between these two groups in relation to preterm birth and weight. However, many of these results found in the studies fall within the non-significant value range. Despite this, the differences for weight and height between IVF/ICSI twins are significant. This indicates that there needs to be a shift away from implanting multiple embryos, which often leads to the birth of twins. Though there is no way to stop an embryo from splitting in utero, it is much less likely to occur and will allow for the birth of a healthier child. Therefore, women should choose single embryo implantation.

Application to Biotechnology
Since IVF is rapidly becoming a commonly used way to treat infertility, it is necessary that couples understand the risks associated with having twins. It is clear that twins conceived through artificial reproduction technologies have worse outcomes; therefore, it is important to increase the amount of singleton births. One way that this can be done is through elective single embryo transfer (eSET). eSET is a procedure in which one embryo, selected from a larger number of available embryos, is placed in the uterus or fallopian tube. eSET helps women avoid several risks to their own health and that of their child that are associated with carrying multiples. There is consensus among experts that the desired outcome of ART is a healthy singleton infant, which is what should be focused on in the future of in vitro fertilization.

Neonatal Outcomes Between IVF/ICSI Singletons and Twins Conceived Naturally and Through Assisted Reproduction

Allyson Brown | The Bishop’s School
Exploring the Potential of Metformin to Prevent the Occurrence of Endometrial Cancer

Natalie Chavarria
Mater Dei Catholic High School

Background

Metformin is a first-line drug, used to regulate the insulin resistance found in type-2 diabetes patients. Recent evidence indicates a correlation between the insulin resistance found in type-2 diabetes patients and the insulin resistance found in endometrial cancer patients. This correlation theories metformin’s potential to inhibit the proliferation of endometrial cancer cells. The theory is currently a controversial topic because evidence concerning the association lacks large, population-based studies confirming the results. This poster will be overviewing four, recently published studies disproving the association between metformin and a decreased occurrence of endometrial cancer.

Abstract

Metformin is a type-2 diabetes drug theorized to inhibit the growth of endometrial cancer, due to the correlation between the insulin resistance found in type-2 diabetes patients and the insulin resistance found in endometrial cancer patients. Evidence confirming this theory remains controversial because large population-based studies are lacking. The following four studies disprove the potential of metformin to prevent the occurrence of endometrial cancer growth. The first study, a 2016 in vivo study, proved the inability of metformin to reduce the ki-67 expression and inhibit endometrial cancer proliferation. The second study, a cohort analysis observing 1,746 subjects from the US healthcare claims between 1995-2011, found no association with metformin and decreased occurrence of endometrial cancer. The third study, a case-control analysis observing 748 subjects from the UK-based General Practice Research Database (GPRD) between 1995-2012, resulted in no association between the use of metformin and a lowered risk of endometrial cancer. The fourth study, observed 748 subjects between 1997-2006 from the utilization databases in Lombardy, Italy, and also found no association between metformin and endometrial cancer occurrence.

Methods and Materials

The first study, an in vivo analysis, involved forty female mice who were divided into two treatment groups at 6 weeks old. Twenty of the mice were fed a high-fat diet (HFD), while the remaining twenty received a low fat diet (LFD). At 10 weeks the two groups were split again. One half of each group received 5 mg/mL of metformin in their drinking water, while the remaining control group received untreated water. At 26 weeks the animals were euthanized. The mice’s uterine tissue was scored for degree of the endometrial hyperplasia (EH), and immunohistochemical staining was used to detect ki-67 expression in the endometrial tissue. The second study observed the 2000-2011 US healthcare claims of metformin users with no prior cancer diagnosis, followed until a diagnosis of endometrial cancer. This involved a total of 272,411 subjects. The third study observed the UK-based General Practice Research Database (GPRD) between 1995-2012. This involved the observation of 1,746 subjects. The exposure, duration and long-term use of metformin was used to determine the association of metformin to an altered risk. The fourth study observed the databases in Lombardy Region, Italy between 1997-2006. The study consisted of 748 subjects and the odds ration in relation to metformin was estimated by the conditional logistic regression model.

Results

The research resulted in a variety of evidence disproving metformin’s association with the prevention of endometrial cancer. The first study, a 2016 in vivo study, confirmed the inability of metformin to reduce ki-67 expression and inhibit endometrial proliferation. The second study, a cohort analysis, observing 272,411 subjects from the US healthcare claims between 2000-2011, found no association with metformin and development of endometrial cancer. The third study, a case-control analysis observing 1,746 subjects from the UK-based General Practice Research Database (GPRD) between 1995-2012, resulted in no association between the use of metformin and a lowered risk of endometrial cancer. The fourth study, observed 748 subjects between 1997-2006 from the utilization databases in Lombardy, Italy, and also found no association between metformin and endometrial cancer occurrence.

Discussion

Research indicates that the ability of metformin to prevent the occurrence of endometrial cancer is currently a controversial topic. Evidence regarding this theory is currently lacking large studies confirming the association. The results of both population analysis and in vivo studies disprove the correlation between metformin and endometrial cancer prevention. This suggests that, evidence confirming the association is insufficient compared to the larger scale of evidence against the association. Therefore, further investigation into the potential of metformin is required.

Application to Biotechnology

The objective of this poster is to present evidence against the association between metformin and endometrial cancer prevention. The evidence against the association was gathered from multiple studies including a recent in vivo study. The biotechnology used to acquire the results from this study include immunohistochemical staining and H&E staining. The steps to Immunohistochemical staining involve: preparing the test sample, retrieving the antigen, blocking the background of the sample, detecting the target and observing the sample. The steps to H&E staining include: staining rehydrated sections of the tissue with Hematoxylin, washing the sample, staining the sample in Eosin solution, washing the sample a second time, dehydrating and observing the sample. This application of biotechnology reveals, through in vivo testing, that the association between metformin and the decreased risk of endometrial cancer development requires further testing.

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References

Advances in Stem Cell Technology Leading to Increased Live Birth Rates in Mice

Molly Demer
San Diego High School

Objective

Development of a reliable method for generating adult fertile mice from induced pluripotent stem cells (iPSCs) is desirable in regenerative medicine because it is regarded as the most stringent test for pluripotency. However, the same technology has vast implications for reproductive science.

Abstract

New applications of stem cell research, more specifically, the use of induced pluripotent stem cells (iPSCs) may provide another avenue to further the progress of reproductive science when gametes are not an option or desired. In 2006, the Yamanaka lab demonstrated that iPSCs can be generated from somatic cells through transfection of four transcription factors known to affect genetic reprogramming of somatic cells back to full pluripotency. This report will explore the methodology of iPSC cell line production and whether the use of a dox-inducible promoter increases the efficiency of live mouse pup births. The Baldwin lab, which utilized the dox-inducible promoter in eight cell lines produced 29 pups from 1,378 injected blastocysts, a 2.1% live birth rate. Their highest producing cell line, IMZ-21, had a 13% live birth rate. The Zhou lab developed six cell lines yielding 27 pups from 1,554 blastocysts, a 1.7% rate of live birth. Cell line IP14D-1 being the most productive with a 3.5% yield. Although the methods used by the Baldwin lab indicate an overall higher efficiency, the difference in yields is not statistically significant. However the highly efficient IMZ-21 cell line should be further investigated for other factors that influence efficiency.

Method and Materials

Generation of iPS cell lines: Mouse embryonic fibroblasts (MEF) were harvested on day 13.5 of pregnancy and prepared for transfection. Retroviral systems that include the Takahashi inscription factors, Sox2, Oct4, c-Myc, Klf4, were used to induce stem cell qualities. Green fluorescent protein (GFP) was used to indicate when the desired traits are expressed. Colonies of fluorescent iPS cells begin to emerge after 10 days, and after 14 days they are isolated for creation of iP Mice. Generation of iP Mice: 2-cell embryos are fused to 1-cell 4N embryo to create tetraploid blastocysts. iP cells are injected into the blastocyst which are then transferred to a recipient mouse. After 17.5 days from transfer, observe the results of matured viable pups. All iP cells and mice were created using similar methods described above with the following exceptions.

Baldwin lab:
Generation of iPSC cell lines: The Takahashi transcription factor gene expression is controlled through the use of dox-inducible promoter which prevents inappropriate expression of these reprogramming factors.

Conclusions

Early work established that embryonic stem cells, once injected into blastocysts could produce live mouse pups. Zhou and Baldwin labs were the first to demonstrate that iPSC cells could also produce live mouse pups. Advances in the methodology for creating iPSC cell lines by the Baldwin lab, that include the use of an inducible promoter system, appear to have a positive effect on the yield of live mouse pups. This is supported by the data (Tables 1 and 2) showing higher overall yield of live pups over multiple cell lines as well as higher efficiency in specific cell lines. The inducible promoter system was used by the Baldwin lab to safeguard against the expression of reprogramming genes that would impede embryonic development. At this early stage of iPSC research it cannot be definitely concluded the differences in efficiency between the two labs is solely due to the dox-inducible promoter system. Other differences in their methods include prolonged valproic acid (VPA) treatment in the Baldwin method and modified cell culture conditions to include knockout serum without antibiotic selection in the Zhou method. Further investigation is required to isolate and test these variables as well as the impact of dox-inducible promoters on a larger scale. Before progressing to other animal models, causes of deformities and infertility needs to be addressed. To best support the goal of reproductive science, iP technology must establish a reliable method of increasing the live birth rate of healthy, fertile offspring.

References


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Relevant Applications to Biotechnology

Most all of the biological reagents necessary to create the iPS cells and the subsequent live organism generated from these iPS cells are products of the biotechnology industry. As new biological tools become available, stem cell research and applications will continue to advance. The goal of producing iPS cells with true pluripotency is to advance regenerative medicine and reproductive science. Within reproductive medicine iPSCs are a method for cloning organisms or a method for making germ cells.
Effects of P-MAPA with Cisplatin on Survivability of Serous Ovarian Carcinomas

Ovarian cancer (OC) is the leading gynecological cause of death in the US, with 80% of patients that survive the first time relapsing within 2 years. 50% of OC cases are ovarian cancers, 50% of which are serous, or fluid-filled, carcinoma. This makes serous epithelial ovarian carcinomas the most common ovarian cancer. Recently, the role of immunity in OC is starting to be studied for development of immunotherapies. It is known that short chain omega-3 palmitoleate anhydride (P-MAPA) is a protein aggregate that is already used in bladder cancer immunotherapy and works by targeting the tumors’ Toll-like receptors (TLR) 2 and 4, which are key in innate immunity and initiate apoptosis and arrested growth.

When P-MAPA was tested against OC in rats, it was successful in reducing tumor growth and survival. However, these results were at 70%, which is substantially higher than the 11.25% response reported in current OC immunotherapies. These results are historically known to increase rates of survival in many cases. This study explores whether P-MAPA can better increase rates of survival with cisplatin (a CIS), a common chemotherapy agent for OC.

Results
Mass and Volume
After 30 days of treatment, rats treated with CIS and CIS+P-MAPA therapy reached 24.4% and 20.3%, respectively, from control OC. P-MAPA therapy had no significant effect on volume yet. After 60 days of treatment, rats treated with CIS+P-MAPA were reduced in tumor size by 16.3%, 41%, and 32%, respectively. At the end of the treatment, CIS and CIS+P-MAPA therapy groups had significant reductions in mass of 30% and 26%, respectively. P-MAPA therapy was not seen to have an effect.

Anatomophotgraphy
Sections were observed between tissue quality in tumors of each group. The control OC group was seen to have tumors of large, dense, solid mass and scattered necrotic spots. The group with P-MAPA therapy had tumors that were smaller and less dense, which resulted in the lowest survival rate. Tumors were very morphologically different, with softer, mobile tissue and very few necrotic spots.

TLR2 and TLRI4 Expression
While TLR2+ was seen to be affected by CIS or CIS+P-MAPA therapies. P-MAPA therapy also upregulated TLR2 expression. The thymus and the combined CIS+P-MAPA therapy did so at the highest extent.

Figure 2. Kaplan-Meier curves showing the time to survival of rats surviving in weeks after beginning of treatment. The P-MAPAC were treated with P-MAPA+cisplatin, the CIS+P-MAPA+cisplatin, and the CIS+P-MAPA were treated with cisplatin. The CIS+P-MAPA+cisplatin therapy also significantly increased lifespan. Figs. 2A, 2B, 2C, 2D, and 2E show the comparison of the effect of cisplatin on various ovarian carcinomas through targeting TLR4 signaling. Journal of Ovarian Research, 6, 1 (2014).

IFN-γ, TNF-α, and IL-6 Levels
The levels of all three molecules (IFN-γ, TNF-α, and IL-6) were previously associated with tumor death were upregulated by the combined CIS+P-MAPA therapy and the P-MAPA therapy. TLR4 and TLR2 expression was increased in tumors of different therapies: represented 0 (absent), 1 (low), 2 (moderate), or n (high). The survival rates of rats treated with CIS and CIS+P-MAPA were significantly lower than the control rats. CIS and P-MAPA therapies as valid treatments of ovarian cancer and P-MAPA therapy has more health promoting effects, such as increased tumor size and stimulating the immune system, than either cisplatin or P-MAPA therapy. Combined CIS+P-MAPA therapy led to the highest expression of TNF-α and IL-6. Compared with P-MAPA therapy, living an average of 65% longer than the control rats. CIS and P-MAPA therapies as valid treatments of ovarian cancer and P-MAPA therapy has more health promoting effects, such as increased tumor size and stimulating the immune system. P-MAPA therapy led to the highest expression of TNF-α and IL-6. Compared with P-MAPA therapy, living an average of 65% longer than the control rats.

Figure 5. Levels of different proteins expressed in tumors of different therapies. P-MAPA therapy was most effective in reducing tumor death. The combined therapy increased the lifespan by about 25%.
It has been proposed that females who undergo assistive reproductive procedures and suffer from recurrent implantation failure (RIF) have abnormal endometrial receptivity. During the mid-luteal phase, the adhesion ligands of the endometrium will become pervasive in the endometrium and inhibitory factors will be removed. A number of molecules have been associated with endometrial receptivity. One such molecule, Mucin 1 (MUC1), is a glycoprotein present abundantly on the endometrial glandular epithelium, is found at a higher level of expression in fertile patients than in infertile patients. Subjects included 14 women with RIF, 25 with recurrent miscarriage (RM), and 20 fertile controls who participated in endometrial biopsy during the implantation window. The spatial and temporal expression of MUC1 was studied using semi-quantitative immunohistochemistry. It was found that MUC1 expression in both luminal and glandular epithelium in women with RIF were significantly decreased compared to that of RM and control groups. This decrease expression was also not found to be associated with demographics or clinical characteristics. Another study investigating MUC1 expression in females with implantation issues suggested that MUC1 expression in an endometrium that differs from fertile and appears to be a consequence of abnormal gene expression. Therefore, it can be strongly supported that decreased expression of MUC1 in endometrial tissues correlates with recurrent embryo implantation failure, and could be a potential target for therapies for enhancing success rates with implantation.

Methods and Materials

The subjects of this study included 59 women <40 years with regular menstrual cycles, typical body mass index, no of current contraception treatments ≥3 months prior to the study. Women with endometrial or uterine pathology were excluded. 14 women were identified with RIF, meaning they failed to achieve a clinical pregnancy post 4 embryo transfers in 3 or more transfers. 25 women were categorized with RM for 3 or more miscarriages prior to 20-week gestation. 20 fertile controls (at least 1 live birth in the last 2-3 years) also participated. All subjects had blood tests from day 9 of the cycle onwards to pinpoint the LH surge. Also, an endometrial biopsy was obtained using a Pipelle sampler (Predimed) or Pipelle Curet (Cook Surgical) at LH+ 7-14 days of the implantation window. The specimens were then immediately washed in phosphate-buffered saline (PBS, pH 7.4) and divided in half. One part was fixed in 10% neutral buffered formalin for immunohistochemistry and the other was examined pathologically for endometrial dating. Samples diagnosed as chronic endometriosis were excluded. The expression of MUC1 in the samples was analyzed through two methods: immunohistochemistry and H-score analysis. Immunohistochemistry: Endometrial specimens were embedded in paraffin wax and cut to a thickness of 4 μm after overnight formalin fixation and serial ethanol dehydration. MUC1 spatial expression in the sample was determined by standard immunohistochemistry: sections were dewaxed in xylene, rehydrated by descending ethanol to PBS, and quenched in 3% hydrogen peroxide in methanol for 20 min. The antigens were then retrieved in a microwave oven with 10mM sodium citrate buffer (pH 9). The sections were then washed in PBS and blocked in the bovine serum albumin buffer for 1 hour at room temperature and incubated at 4°C overnight with mouse monoclonal anti-human MUC1 antibody. Next, the sections were washed in 0.5% PBS. The antibody binding was visualized by peroxidase substrate 3,3′-diaminobenzidine tetrahydrochloride and counterstained with hematoxylin. The sections were then dehydrated, put in synthetic resin DPK, and examined under light microscopy. H-score analysis: MUC1 expression intensity was quantified through the equation H-score = ΣPi, where i was the stained intensity (0 = negative, 1 = weak; 2 = moderate; 3 = strong) and Pi was referred as percentage of cells stained at each intensity (0–100%). H-score was obtained in 5-qualified 40x visual fields per sample and scored by two independent observers.

Results

The results of the experiment effectively demonstrated the correlation between decreased expression of MUC1 in endometrial tissues and recurrent embryo implantation failure. Demographic details such as age, number of previous pregnancies, number of live birth and previous miscarriage, BMI, and menstrual cycle length seemed to have no significant correlation to MUC1 expression. MUC1 demonstrated strong immunoreactivity in both the luminal and glandular epithelial cells of the control group. However, it was much less intense in the RM subjects and extremely low in the RIF subjects. H-score analysis results demonstrated that the expression of MUC1 in luminal and glandular epithelium in RIF subjects was significantly below that in the control and RM subjects. A multivariate linear regression model of demographic and clinical characteristics shows data as follows: MUC1 H-score in luminal epithelium post-hoc statistical power (P = 0.05) was 0.93. MUC1 H-score in glandular epithelium post-hoc statistical power (P = 0.05) was 0.89. H-score analysis results suggested that it will adhere to endometrial lining and begin to develop. Unfortunately, during this period, some women’s may suffer from recurrent implantation failure. This leads to an unfavorable result in pregnancy and is also used to study biomarkers and differentially expressed proteins in biological tissue. H-score analysis allows researchers to manipulate the test results from the immunohistochemistry test. It measures staining intensity amongst a variety of other factors. This H-score is easily calculated by computer allowing scientists to gain access to a wide analysis of data in a matter of minutes. Scientists are now one step closer to helping women who suffer from recurrent implantation failure and hopefully assist them in their journey of pregnancy. Finally, the results indicate that expression of MUC1 is decreased in the endometrial tissues of women with RIF, and it will be able to allow patients’ MUC1 levels to be quickly tested prior to implantation.

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I would like to extend a special thanks to Dr. Ericka Sweeney-Mitchell for all her support and guidance throughout the research process. Her positive energy and enthusiasm for science was incredibly inspiring. Also, I would like to thank Dr. Kelly Church for her guidance and insight with the written portion of this poster. Furthermore, I am extremely grateful for the time Dr. Mei-Chen Chang, Dr. Irene Su, and the other presenters of the Academy. Finally, I would like to acknowledge my fellow ROSA sisters for making this Academy such a wonderful and memorable summer experience.

References


A variety of applications and services were used during this study. The hypothesis of the study investigates the correlation of MUC1 expression with recurrent implantation failure. embryonic implantation is a crucial part of a assisted reproductive techniques that involves the injection of a fertilized egg directly into the uterus in hopes that it will adhere to endometrial lining and begin to develop. Unfortunately, during this period, some women may suffer from recurrent implantation failure. This leads to an unfavorable result in pregnancy and is also used to study biomarkers and differentially expressed proteins in biological tissue. H-score analysis allows researchers to manipulate the test results from the immunohistochemistry test. It measures staining intensity amongst a variety of other factors. This H-score is easily calculated by computer allowing scientists to gain access to a wide analysis of data in a matter of minutes. Scientists are now one step closer to helping women who suffer from recurrent implantation failure and hopefully assist them in their journey of pregnancy. Finally, the results indicate that expression of MUC1 is decreased in the endometrial tissues of women with RIF, and it will be able to allow patients’ MUC1 levels to be quickly tested prior to implantation.
Abstract

Ovarian cancer (OC) is the fifth leading cause of death in women and the leading gynecological disease that results in death. This is due to inadequate diagnostic markers for women who have undergone late detection and minimal early symptoms. If detected in early stages, OC patients have a 90% or greater chance of survival; whereas in later stages, the survival rate dwindles to a mere 30%. Additionally, recurrence rate of ovarian cancer detected at stage I is only 10% while at stage 3 it is a whopping 50%. The identification of a potential prognostic marker provides key information aiding early detection. Currently, CA-125 tests are occasionally used for early detection, however, prove unreliable due to its elevated levels in early stages - creating the necessity of a new early detection marker. RHAAM overexpression has been correlated with metastasis as well as the promotion of an invasive phenotypic expression in several cancers, including OC. In normal tissue, RHAAM has been known for its role in miosis and cell growth. RHAAM expression has been studied through urinelsis, ELISA, and tissue staining in normal patients and OC patients. RHAAM antibodies were used in order to assist in detection of the protein through the aforementioned tests in tissue and urine samples. The result found that patients with OC exhibited elevated tissue RHAAM levels, while staining intensity increased with escalating cancer stage and tumor grade. Additionally, urinary RHAAM levels were elevated in all patients. In all control patients, urinary RHAAM levels were undetectable, while 6/9 OC patients exhibited elevated RHAAM levels. Furthermore, urinary RHAAM in women with OC patients had a significantly higher level than OC patients. When measured in OC cells, RHAAM levels were 20-70 times higher in control ovarian cells. Using RHAAM as a prognostic marker for OC is highly effective and exhibits increased reliability to current methodologies. The use of RHAAM as a prognostic marker can revolutionize early detection, as it is noninvasive, reproducible, and relatively inexpensive.

Methods and Materials

Objective

About 20,000 women will be diagnosed with ovarian cancer this year, while around 14,000 will die at the hands of it. Currently, CA-125 tests are the only prognostic markers used in the detection of ovarian cancer, however, this test only works 50% of the time and gives false positives for benign gynecological diseases causing most physicians to shy away from using it. Due to the inadequacies of current methodologies, ovarian cancer is oftentimes detected at later stages, decreasing potential survivability by over 60%. The niche of reliable diagnostic markers for ovarian cancer has yet to be filled until now, with the analysis of hyaluronan mediated motility receptor (RHAAM) overexpression. This novel research can catapult the creation of a prognostic marker and has the potential to become routine at clinical checkpoints, saving thousands of lives every year.

Experiment 1: First, researchers sought to determine the potential correlation between RHAAM expression and ovarian cancer. To do so they analyzed RHAAM levels in tissue samples from a 27 patient cohort as well as used cell lines to confirm the results. The patient cohort included ovarian cancer patients varying from stages I-V as well as normal patients. Tissue samples of ovarian cancer came from patients who had tumor excision through surgery and controls were used from women who had their ovaries removed or oophorectomy due to unrelated diseases. The patient cohort were from varying stages of ovarian cancer. Additionally, excised tissue was examined and reviewed to confirm diagnosis by cross checking with the guidelines published through the International Federation of Gynecology and Obstetrics. The tissue was formalin fixed and embedded in paraffin in order to increase storage potential. Subsequently, immunohistochemical studies were performed on the tissue samples. The samples were immunostained with Rabbit Anti-Human CD168 antibody, dripped, and mounted. Staining intensity was then monitored and localized to the specific regions in the cell.

Experiment 2: The link between ovarian cancer and RHAAM overexpression was then analyzed in cell lines to confirm the results. Cell lines were cultured in complete media and western blotting was used to analyze expression of RHAAM protein. OC cell line was compared to normal cells which originate from normal human surface epithelium. Anti β-actin was used as a control because of its expression in all endocytic cells while RHAAM antibody was used at a 1:1000 dilution and protein bands were monitored.

Discussion

In all, RHAAM has proven to be correlated with ovarian cancer progression from the earliest to the latest stages. RHAAM expression has been elevated in ovarian cancer patients through tissue as well as urinary analysis. In the future, similar studies need to be conducted in larger patient cohorts to confirm the results. Western blotting and ELISA tests have proven reliable methods to test elevated RHAAM and protein levels in urine allowing it to be used for prognostic testing in hospital labs around the nation. Furthermore, CA125 tests have proven unreliable on their own with a low detection rate, and high rate of false negatives - indicating unnecessary stress and procedures on patients. However, when the study looked at elevated RHAAM and elevated CA125 together to detect ovarian cancer, they had success in 96% of patients. While RHAAM testing worked in 26/29 patients and sole CA125 worked for only 19/29 patients showing that RHAAM detection helps to optimize early detection.

This research represents a groundswell for a prognostic marker to be created through the use of a RHAAM and immunoaffinity chromatography for more accessible use that will decrease the rate of early detection and ovarian cancer treatment to be transformed.

Relevant Applications to Biotechnology

The relevance and significance of this research lies in its potential as a prognostic marker. Similar to a pregnancy test, a RHAAM test could be formatted using strip tests. This antibody was used, with success, in all western blots and ELISAs in this study. When soaked in patient urine, the test would use immunoaffinity chromatography and change color; it would also be much less expensive. To work through RHAAM binding to the antibodies present. This can revolutionize early screening and detection methodologies for ovarian cancer as it makes detection accessible and affordable.

Acknowledgements

I would like to thank Dr. Ericka Senegar-Mitchell, Dr. Chang, and Ms. Patricia Winter for helping organize this program and giving us the opportunities of a lifetime. I would also like to give a special thanks to Dr. Zuzana Hostomska who believed and invested in my idea from the start, and Dr. Jamie Turley, E.A. (2007). The hyaluronan receptors cd44 and rhamm (cd168) form complexes with ptk7, which maintain high basal motility in breast cancer cells. The Journal of Cellular Biology, 28(22), 1667-1680.

References

Incidence of Ovarian Hyperstimulation Syndrome in IVF Patients Using Kisspeptin 54 for Ovulation Induction

Victoria Li

Canyon Crest Research

Background

Human chorionic gonadotropin (hCG) is commonly used in in vitro fertilization (IVF) protocols to induce ovulation. However, some patients may develop Ovarian Hyperstimulation Syndrome (OHSS) after hCG administration, characterized by the enlargement of ovarian follicles, ascites, pleural effusion, and hyperviscosity. Previous studies have shown that kisspeptin, a neuropeptide, can stimulate ovulation and inhibit the release of hCG. This study aimed to evaluate the efficacy and safety of kisspeptin 54 in the treatment of OHSS.

Methods and Materials

Participants

This was a randomized, double-blind, placebo-controlled trial involving 404 patients with severe OHSS. The study was divided into two groups: the kisspeptin group (205 patients) and the placebo group (203 patients). Patients were assigned to either group based on their OHSS grade.

Results

The results showed that kisspeptin significantly reduced the incidence of severe OHSS compared to the placebo group. Patients in the kisspeptin group had lower levels of serum anti-Müllerian hormone (AMH), a marker of ovarian reserve, and a lower risk of developing severe OHSS.

Conclusion

Kisspeptin 54 appears to be a promising option for the prevention of OHSS in IVF patients. Further research is needed to establish its safety and efficacy in a larger, more diverse patient population.

Applications to Biotechnology

In all patients, IVF requires specialized machinery and other assisted reproductive technology (ART). IVF physicians and imaging technicians used both pelvic and transvaginal ultrasound extensively to ensure proper follicular growth and ovulation. IVF also requires fresh embryos to be transferred to the uterus within 24 hours of ovulation.

Acknowledgements

I want to thank Dr. Ericka Senegar-Mitchell and all my other ROSA sisters for inspiring me in ways beyond science and making my summer so much more than I could have imagined. Thank you to Dr. Jeffery Chang, Dr. Irene Su, and our other guest lecturers for taking the time to speak to us and for giving us small windows into your life and work. A special thank you to Patrice Winter and everyone else at the Ovarian Cell Consortium for making this entire program happen and allowing me to have this amazing opportunity. And of course, thank you to my family and friends for their endless support and encouragement.

References


Teratogenic Effects of Immunosuppressive Drugs Given to Expectant Mothers after Organ Transplantation

Kendall Ota

Objective
Each year, over 10,000 transplantation surgeries are performed on women. While these transplantations save and improve an individual’s quality of life, they also require the patient to take immunosuppressive drugs, which help to reduce chances of organ rejection, for the rest of their lifetime. This poster will evaluate whether there is an increased risk of congenital defects and other serious complications as a result of immunosuppressive drugs, such as antiproliferative agents, given to women during their pregnancies.

Abstract
Absolute uterine factor infertility affects approximately one in every 500 women. For years, only the options for these women were adoption and gestational surrogacy. Recent advances in uterine transplantation offer the opportunity for infertile women to carry and give birth to infants.1 However, uterine and other organ transplantations necessitate immunosuppressive drugs (ISDs) in order to minimize graft rejection. ISDs can result in intrauterine growth restriction, congenital defects, and higher miscarriage rates of up to 48%. 2 In order to evaluate the risk of these fetal anomalies, data was collected from several studies measuring the extent of impact ISDs given to expectant mothers have on developing fetuses. Studies tested the exposure of different ISDs, particularly antiproliferative agents, at various points in the pregnancy of both women and animal models. Researchers separated the drugs into categories of high, medium, low, and unknown risk, and detailed each ISD’s embryolethality, teratogenicity, and effect on fertility.3 Mycophenolate (MMF) presented an increased risk of miscarriage (32-45%, comparable to the general population risk of 15-20%) and birth defects (26%, comparable to the general population risk of 3%). Out of 77 patients exposed to MMF, 28 reported miscarriages and 14 structural malformations.4 Azathioprine (AZA) and 6-mercaptopurine (6-MP), however, were considered to be generally safe; 155 pregnant women exposed to these drugs were surveyed, and there was found to be no statistical difference in conception failures, birth defects, or spontaneous abortion, though researchers strongly suggest additional ultrasound monitoring in these pregnancies.5 Dosages of immunosuppressive medications should be tailored for conception plans in order to maintain efficacy while minimizing fetal risk.6 The results from these studies can be applied toward helping women who have undergone organ transplantation, allowing the increase in possibility for infertile women to carry and give birth to children through uterine transplantation.

Materials & Methods
Researchers reviewed the health effects of antiproliferative ISDs on pregnant women and developing fetuses. Animal models and retrospective observational studies were utilized out of ethical concerns.7 77 pregnancies exposed to MMF, 155 pregnancies exposed to AZA and 6-MP, and 16 pregnancies exposed to sirolimus (SLM) were monitored, in addition to animal models. Individual cases of exposure were also reviewed, including that of one pregnant woman to 50,000 mg of MMF and later miscarriage. These ISDs were evaluated for gametogenesis, mutagenesis, teratogenesis, and deleterious effects on pregnancy; researchers calculated the percentage rate of each risk for each drug. Based on these results, the drugs were sorted into categories of high, medium, and low risk.

Results & Interpretation

<table>
<thead>
<tr>
<th>Immunosuppressive</th>
<th>Gametogenesis</th>
<th>Mutagenesis</th>
<th>Teratogenesis</th>
<th>Pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mycophenolate</td>
<td>&lt;i&gt;No effect on fertility&lt;/i&gt;</td>
<td>Mutagenic in vivo</td>
<td>Clastogenic, teratogenic in vivo</td>
<td>Crosses placenta +</td>
</tr>
<tr>
<td>Azathioprine</td>
<td>No present risk</td>
<td>Mutagenic</td>
<td>Carcinogenic and teratogenic in vivo</td>
<td>No present risk</td>
</tr>
<tr>
<td>Sirolimus</td>
<td>No present risk</td>
<td>No present risk</td>
<td>3 miscarriages and one child with multiple malformations (mycophenolate)</td>
<td>No present risk</td>
</tr>
</tbody>
</table>

Risk Factor

Figure 2: Figure 2 (above): Outcomes of the Meta-Analysis of Antiproliferative Immunosuppressive Drugs. The table above shows the study’s findings on the effects of mycophenolate, azathioprine, and sirolimus. Adapted from tables by Levy, C., Rigo, J.-M., Lerox, M., Decanter, C., Le Mauphan, K., Parent, A.-S.,…Vantyghem, M.-C. (2015).

Figure 3 (left): Evaluation of Risk of Deleterious Effects on Fetal Development. The table demonstrates the relative risk of harmful effects such as miscarriage or birth defects of immunosuppressive agents on developing fetuses. Adapted from results by Levy, C., Rigo, J.-M., Lerox, M., Decanter, C., Le Mauphan, K., Parent, A.-S.,…Vantyghem, M.-C. (2015).

Discussion
In evaluating the risks of antiproliferative ISDs, increases in gametogenesis, mutagenesis, teratogenesis, miscarriage, and infertility indicate high risk factors. Drugs with high risk (like MMF) should be contraindicated in the pre-conception period (the 3 1/2 months before pregnancy) and during pregnancy. Drugs considered to be medium risk (such as SLM) should be used with caution; even low risk drugs (like AZA or 6-MP) should only be taken with additional ultrasound monitoring and if completely necessary. Continuing high-risk ISDs can result in negative health impacts on both child and mother. The management of patients using ISDs who wish to become pregnant should involve consultations to change drug dosages in order to reduce fetal risks, patient-doctor planning of the pregnancy, and careful monitoring. It is crucial that the patient be advised of the risks and for the patient to employ effective contraception until pregnancy is desired.

Relevant Applications to Biotechnology
Over the past twenty years, over 730,000 organ transplantations have been completed. Many of these transplant patients can go on to lead healthy lives. Understanding the risks of ISDs on pregnancies can help with these patients’ future success. Additionally, the past year has seen many advances in uterine transplantation. Although it is still in its developing stages, uterine transplantation would allow previously infertile women to naturally carry and give birth to children. Since immunosuppressive medications are an automatic risk in these women’s future pregnancies, it is imperative to better understand possible pregnancy and fetal development complications in order to ensure favorable outcomes in uterine transplantation.

Acknowledgments
I would like to thank Dr. Ericka, Dr. Chang, Ms. Winter, and the many other doctors and professionals who dedicated their time and efforts in ensuring this program’s success; Dr. Church, Katte Larruri, and my RSA sisters for their support and assistance; and my family and friends for their love and encouragement.

References

Figure 1 (above): Chemical Compounds. The chemical compounds of (in clockwise rotation) mycophenolate mofetil, sirolimus, and azathioprine are illustrated. Adapted from PubChem.

Figure 2 (above): Outcomes of the Meta-Analysis of Antiproliferative Immunosuppressive Drugs. The table above shows the study’s findings on the effects of mycophenolate, azathioprine, and sirolimus. Adapted from tables by Levy, C., Rigo, J.-M., Lerox, M., Decanter, C., Le Mauphan, K., Parent, A.-S.,…Vantyghem, M.-C. (2015).

Figure 3 (left): Evaluation of Risk of Deleterious Effects on Fetal Development. The table demonstrates the relative risk of harmful effects such as miscarriage or birth defects of immunosuppressive agents on developing fetuses. Adapted from results by Levy, C., Rigo, J.-M., Lerox, M., Decanter, C., Le Mauphan, K., Parent, A.-S.,…Vantyghem, M.-C. (2015).
Objective

Since cancer survival rates are increasing each year, there are a larger amount of infertile women as a result of gonadotoxic chemotherapy treatment. A common condition linked with infertility is premature ovarian failure (POF), which is the loss of normal ovarian function before age 40. Currently, there are some ways this devastating condition can be treated, but it would be more beneficial to patients’ quality of life if it could be prevented. There have been promising results for the use of the drug gonadotropin-releasing hormone agonist (GnRHa). By suppressing ovarian function while chemotherapy is received, primordial follicles could be saved from being damaged, which preserves fertility for women.

Materials and Methods

There have been several trials done in the last decade that suggest that GnRHa could prevent POF. Each included women of relatively normal ovarian function and who were prescribed chemotherapy. In this study, each patient was randomized to either GnRHa or chemotherapy alone, in order to see if they did not have previous chemotherapy, radiotherapy, or other cancers. Both of the following trials were performed to understand the extent of fertility preservation that the administration of GnRHa has, which included the prevention of POF.

Trial 1: This randomized phase 3 trial was conducted at 16 Italian health centers in 2009. It included 281 breast cancer patients, of varying stages, who were of median age of 39. Each patient was randomly assigned to a group, making 148 patients in the group that would receive GnRHa with chemotherapy and 133 in the group that would receive chemotherapy alone. The GnRHa group was given an intramuscular dose of 3.75 mg at least one week before the start of chemotherapy and then every four weeks for the duration of treatment. The type of chemotherapy used was cyclophosphamide. Menstrual activity and FSH and estradiol levels were assessed for one year after the end of chemotherapy.¹

Trial 2: Another similar study was conducted in 2011 by the American Society of Clinical Oncology. This consisted of 129 Hodgkin or non-Hodgkin lymphoma patients, with a median age of 25.6 years, within a multicenter setting. Patients were randomly assigned to the group that would receive GnRHa with chemotherapy, which was 65 women, or the group that would receive only chemotherapy, which was 64 women. In the GnRHa group, the patients were given 11.25 mg of GnRHa by intramuscular injection every 12 weeks, with doses beginning 10 days before the start of chemotherapy. The treatment included alkylating agents containing chemotherapy and patients had to be at least 8 cycles of chemotherapy in order to produce high levels of gonadotoxicity for the trial. The goal was to assess the rate of POF in each group after one year of follow-up, in addition to levels of FSH and estradiol levels.²

Results

After analyzing these studies, it is possible that GnRHa could be used to prevent POF and preserve fertility for women undergoing chemotherapy. Although, some results were inconsistent, so further trials are needed.

Trial 1: In this group, 21 patients were considered unevaluable due to unrelated conflicts with the chemotherapy administration. Of the remaining 260 women, in the GnRHa group, only 8% suffered POF, whereas in the control group, 26% suffered POF. In the GnRHa group, also, rates of early menopause were lower and resumption of menses was higher, along with twice as many pregnancies as the control group.³

Trial 2: By the one year follow-up, there were 45 patients in the GnRHa group and 39 in the control group. There were two patients who faced severe adverse effects from the GnRHa group, but there is no confirmed correlation. Fortunately, most patients had recovered normal ovarian function following treatment, except for 20% and 19% of women in the GnRHa group and control group, respectively, who had developed POF. Additionally, two patients in the GnRHa group had pregnancies. The proximity of this trial’s results raise awareness that GnRHa may have inconsistent effects, although this form of treatment has a longer-term benefit on fertility that allows women to maintain normal ovarian function, but it has not been concluded.⁴

Discussion

To conclude, GnRHa has demonstrated that it could become a common solution for preventing POF in women receiving chemotherapy. Numerous trials have shown that GnRHa decreases POF in various ages of women and diseases, making it a versatile option. This could lead to the administering of GnRHa becoming an accepted technique in the field and if the quality of the drug could be approved to increase outcomes of POF prevention, it could be used instead of embryo cryopreservation or other current techniques. Although, some results have been inconsistent and the long-term maintenance of ovarian function as a result of this drug is not certain, so further studies will be needed to prove whether GnRHa could eventually be used routinely. Trials consist of various diseases, ages, and dosages will be most beneficial to see how this drug varies in each case, because much of that information is not known. If this option of fertility preservation could be perfected, women would not have to use expensive techniques in order to have children following chemotherapy.

Abstract

Many chemotherapy drugs can be extremely damaging to fertility, especially in a woman’s ovaries.⁵ The following research offers insight on a feasible way for premature ovarian failure (POF) to be prevented, ultimately preserving fertility while undergoing chemotherapy. Gonadotropin-releasing hormone [GnRH] is produced in the hypothalamus that signals to the pituitary gland to make FSH and LH, which are sent to the ovaries to produce estrogen and progesterone and control follicular recruitment.⁶ The agonist of this hormone has the potential to protect the ovaries during chemotherapy, by temporarily suppressing ovarian activity. When the ovaries are not growing follicles, chemotherapy drugs are not able to damage this process to a full extent.⁷ In one study, 146 patients were given GnRHa along with chemotherapy, and in a control group, 71 patients did not receive chemotherapy. Two years later, it was observed that only 13% of the group given GnRHa suffered POF, whereas in the control group, 51% suffered POF.⁸ Also, in the GnRHa group, there were 123 healthy newborns, compared to 96 in the GnRHa group.³ This study focuses on the preservation of fertility, which preserves fertility for women undergoing chemotherapy. Although this is not the most common form of fertility preservation, the use of this drug should be considered for women who are receiving chemotherapy and interested in having children later on. Using GnRHa during chemotherapy would eliminate the need for fertility preservation.¹ This poster template is 36” high by 48” wide. It can be used to print any poster with a 3:4 aspect ratio.

Effectiveness of GnRHa in Preventing POF

Applicatins to Biotechnology

In the emerging field of oncology, there are many exciting discoveries being made using new techniques and procedures. This specific research would not be possible without the engineering of GnRHa, a peptide, which is able to influence the endocrinology and target GnRHa receptors in patients. By using recombinant DNA technology, quality drugs, such as GnRHa, are being made to improve the health and quality of life of many patients. Revolutionary technology like this is also making the manufacturing of drugs more efficient by identifying problems that could cause them to fail early on, even before the clinical stage. Because of this, drugs like GnRHa could be improved and prevent infertility caused by gonadotoxic chemotherapy. These advancements are changing the way patients are being treated and given access to new medications, leading to a more promising future of fertility.

Acknowledgements

First of all, I would like to thank Dr. Ericka Senegar-Mitchell for her encouragement through this experience and for inspiring me to be a strong woman in science. Also, thank you to Dr. Chang for teaching us about this amazing field of medicine, Ms. Winter for making this program such a life-changing experience, and especially my OSA sisters for being there for me through it all. Lastly, I am so grateful for my wonderful parents who show their endless love and support for me always.

References

Background

One in every 7 women will develop ovarian cancer in her lifetime, and even with efforts to improve chemotheraphy and surgery options, existing treatments have low long-term success rates.2 However, recent advances in immunotherapy, particularly with chimeric antigen receptor (CAR) T-cells, deaths due to ovarian cancer (OC) in the US.3 Thus, efforts to improve CAR therapy have brought a superior alternative to chemotherapy, as it harnesses the power of the patient’s immune system to target cancerous cells exclusively, thus limiting damage to healthy tissues.4 Within CAR T-cell therapy, in hematologic cancer has been successful, results with solid tumors have been underwhelming, in part due to the on-target, off-tumor effects.1 Inhibiting H8 CAR T-cell therapy in healthy tissue. This poster will explore the efficacy of the antigen 5T4 as a CAR T-cells used to treat ovarian cancer.

To create the 5T4-specific CAR T-cells, T-cells were isolated from patient blood using a protocol from in vitro experiment. The cells were then co-cultured with one of three lentiviral vectors: 3 μl of H8 CAR, 4 μl of 2E4 CAR, or no vector. The T-cells were counted every other day, beginning on day three, and divided for 14 days.

Abstract

In 2018, there are projected to be more than 120,000 new cases and 14,000 deaths due to ovarian cancer (OC) in the US.3 Thus, efforts to improve CAR therapy have brought a superior alternative to chemotherapy, as it harnesses the power of the patient’s immune system to target cancerous cells exclusively.1,2 While the therapy’s success with hematologic cancers hope for solid tumors, unique problems such as on-target, off-tumor effects must be addressed.1-3 ST4-antigens may target CAR for T-cells as they are overexpressed in ovarian tumors, but have been limited expression on normal tissues. The goal of this study was to conduct a series of tests to determine the utility of ST4-antigens as a target for CAR T-cells in OC. Recently, scientist transformed two anti-ST4 CAR, varying in affinity to 5T4, into T-cells taken from 12 patients. Then, they were co-cultured with them their cytotoxic ovarian tumor. After 24 hours the supernatant was collected and INFγ and IL-2 levels assessed. Results suggested a correlation between amounts of INFγ and IL-2 secretion and ST4 expression, with all values ≥0.65, indicating immune activation. In another experiment, NSG mice were injected with ST4-CAR T-cells. At day seven, those with tumors received varying doses of anti-ST4 CAR T-cells, mock-transduced T-cells, or saline, then had their tumor size monitored at regular intervals. Mice given ≥106 H8 CAR or a placebo died within 90 days while mice given ≥106 H8 CAR had a 100% survival rate past 100 days.1 This establishes ST4 as a promising target for CAR T-cell therapy in OC, and potentially for other solid tumors that express ST4. However, before advancing to clinical trial, ST4’s on-target, off-tumor effect must be confirmed in further studies.

Materials and Methods

Two ST4-specific monoclonal antibodies, a higher affinity H8 CAR construct and a lower affinity 2E4 CAR construct, was determined by Biacore, which measures the concentration of the antigens on a sensor surface as they bind and dissociate.5 SCOV-3 and OVCA-3 ovarian cancer cells line, transfected to express luciferase (for use with bioluminescence imaging later), were also obtained from Dr. Valabrega. Both cell lines express 5T4, respectively. The cell lines were cultivated in a single layer and routinely checked for contamination.6

Results

1. Taken together, these three experiments demonstrate the efficacy of ST4 as a target antigen for CAR T-cell therapy. The first experiment supports the notion that there is a high level of expression in ST4 in ovarian cancer cells. The second study suggests that the higher affinity H8 construct may be the most effective at eradicating the remaining cancer cells. The third study demonstrates that the H8 CAR construct confers a significant survival advantage to mice with ovarian tumors. Finally, the CAR T-cells can be used to target autologous tumors. A final study concludes that this treatment is efficacious for helping me focus my research and actualize my dreams for this poster. I would also like to acknowledge Dr. Chang, Dr. Su and all our other guest lecturers for so generously donating their time to this program. Finally, thanks to all of my ROSA lecturers whose support and love has made this summer one to remember.

Acknowledgements

References


4. This indirect staining method provides a greater sensitivity to the stain. Experiment 2 used enzyme-linked immunosorbent assay (ELISA) to measure amounts of INFγ and IL-2 produced by each sample. Finally, experiment three used biotechnology to help measure tumor burden in mice. Before the experiment began, the SCOVA-3 Ccells were transduced with a deactivated adenovirus that expresses the 5T4 antigen, allowing for tumor size to be assessed via bioluminescence.6

Discussion

This study shows for the first time that ST4-specific CAR T-cells can produce an immune response in autologous tumors. ST4-specific CAR T-cells were also found to confer a statistically significant survival advantage on affected mice, especially in doses ≥10×10^6. This study also confirms previous findings that the ST4 antigen is highly expressed in majority of ovarian tumors, and suggests that it may be able to minimize on-target off tumor effects. When taken together, these experiments strongly support the validity of targeting ST4 as a new CAR T-cell therapy’s success with hematologic cancers gives hope for solid tumors, patient’s immune system to target cancerous cells exclusively, thus limiting damage to healthy tissues.1-3 While the therapy’s success with hematologic cancers hope for solid tumors, unique problems such as on-target, off-tumor effects must be addressed.1-3 ST4-antigens may target CAR for T-cells as they are overexpressed in ovarian tumors, but have been limited expression on normal tissues. The goal of this study was to conduct a series of tests to determine the utility of ST4-antigens as a target for CAR T-cells in OC. Recently, scientist transformed two anti-ST4 CAR, varying in affinity to 5T4, into T-cells taken from 12 patients. Then, they were co-cultured with them their cytotoxic ovarian tumor. After 24 hours the supernatant was collected and INFγ and IL-2 levels assessed. Results suggested a correlation between amounts of INFγ and IL-2 secretion and ST4 expression, with all values ≥0.65, indicating immune activation. In another experiment, NSG mice were injected with ST4-CAR T-cells. At day seven, those with tumors received varying doses of anti-ST4 CAR T-cells, mock-transduced T-cells, or saline, then had their tumor size monitored at regular intervals. Mice given ≥10^6 H8 CAR or a placebo died within 90 days while mice given ≥10^6 H8 CAR had a 100% survival rate past 100 days.1 This establishes ST4 as a promising target for CAR T-cell therapy in OC, and potentially for other solid tumors that express ST4. However, before advancing to clinical trial, ST4’s on-target, off-tumor effect must be confirmed in further studies.

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Acknowledgements

References

Objective

12% of people in the US are struggling with infertility; the solution is Assisted Reproductive Technology (ART), which uses different technologies to try and help couples conceive a child. Some of the methods used include: In Vitro Fertilization (IVF), surrogacy, and donor eggs. However, many of these techniques are very costly; for example, IVF can cost on average about $11,500. Many couples can not afford to pay for assisted reproductive care because it is not covered by health insurance, due to the cost of the procedure and drugs. The goal of this research is to determine why insured women do not continue their IVF treatment.

Methods and Materials

The patients from this study went to a private academically affiliated infertility center called Boston IVF for their IVF treatment. There were 893 patients, from the ages of 18-42, that were all insured, completed one IVF cycle but didn’t return for at least one year, and did not achieve a live birth. 312 women were emailed an online survey which asked them about whether they had continued their IVF treatment, if they continued elsewhere, why they discontinued the treatment, and what could have made their experience better.¹

Results

In this study with 893 infertile and insured women who had finished one cycle of IVF, only 312 women completed the survey, demonstrating that two-thirds of the participants stopped their treatment and did not seek further care. When they were asked why they discontinued their treatment 40.2% said that the process was too stressful, 25.1% said that they couldn’t afford the costs, and 24.6% said that they had lost their health insurance coverage, almost 50% of participants stopped treatment due to financial issues. In addition, it has been shown that infertile people often have psychological issues, like depression and anxiety. When participants were asked for feedback almost 40% said that they wished they had a mental health professional to help them through this process.²

Conclusions

IVF is not accessible to everyone due to the price of the procedure and the medications needed. There are many different models in other countries that offer solutions to this issue, for example, Australia, New Zealand, and Israel. Australia uses public health insurance and ART. The cost of ART is about $10,000 but if you have Medicare, Australia’s national health insurance, they offer a partial reimbursement of $6,000-$7,000. New Zealand offers access to free ART, however, it depends on the women’s age and body mass index (BMI). Israeli also follows a similar model because they have a national health insurance which covers the full IVF cost for women under the age of 45 until she has up to two kids with her current partner. Although, Israel covers the full cost of IVF, fertility rates are declining, in 2007 18% of Israeli women achieved a live birth through IVF, and three years later it became 14.8%.³ However Australia and New Zealand have more successful live birth rates, during the first cycle the cumulative live birth rate was at 32.7% and by the eighth IVF cycle 54.3% of women achieved a live birth. ³ If we apply the different ART coverage methods used in countries, like Australia, New Zealand, and Israel, to countries struggling with infertility, ART can be available to all. Even though, not all of the women in these countries achieve a live birth, they all have the equal opportunity to conceive.

Application to Biotechnology

Assisted Reproductive Technology uses technology to help a woman conceive a child. IVF is a type of ART that uses technology because it involves extracting egg and sperm and physically fertilizing the egg on a petri dish. In order to extract the eggs, the physician uses ultrasound and guides the needle to the ovary where it retrieves mature egg from the surface of the ovary.

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References

Congenital Malformations in Children Conceived by In Vitro Fertilization and Intra-cytoplasmic Sperm Injection

Objective

Children conceived through IVF and ICSI have an increased chance of many chronic and/or life-threatening illnesses and congenital malformations. The goal of this research is to emphasize the importance of finding causes and preventions of these abnormalities during fetal and infant development.

Abstract

About 10% of women and 17% of men have infertility. In Vitro Fertilization (IVF) and Intra-cytoplasmic Sperm Injection (ICSI) are the two most common methods in assisted reproduction. The first successful birth through IVF occurred July of 1978 and the first successful birth through ICSI was January of 1992. IVF and ICSI have helped many couples with fertility and conception problems, as well as other health issues. Five million babies have been born through IVF and ICSI, but not all of the children were born without complications and health issues. IVF and ICSI increase the chances of the child being born with congenital malformations, chronic illnesses, and developmental issues by 4.2.6 In a study in Belgium of 5,884 infants born through IVF and ICSI, cardiac malformations were found to be the most common.1 Out of 281 IVF and ICSI born children in a study in the United States, 1.27% had septal heart defects, as pulmonary stenosis (obstruction of blood flow from right ventricle to pulmonary artery) and ventricular septal defect (abnormal opening in the heart between the lower ventricles).5 In a meta-analysis of twenty-four studies, 74,644 children born through IVF and ICSI had a 2.01% increased risk of malformations in the nervous system, 1.66% in the digestive system, and 1.64% in the cardiovascular system.7 These studies demonstrate the risk of IVF and ICSI and the chance of abnormalities and chronic illnesses in children conceived through these two processes. A significant cause of the malformations in the children has not been identified and no processes have been found that prevent these abnormalities.

Materials and Methods

In the Netherlands, data on congenital malformations in children conceived naturally and through assisted reproductive technology is collected from national professional perinatal and neonatal registers: the National Perinatal Database for Primary Care, a register of midwife-assisted births; the National Perinatal Database for Secondary care, register of obstetrician-assisted births; and the National Neonatology Database, carried out by pediatricians. In the National Perinatal Database, all birth records of children conceived through spontaneous pregnancies were selected to constitute a control group (n=314,605) by excluding all pregnancies where the use of assisted reproduction was coded. The IVF study population consisted partially of a cohort of 1935 IVF children, 9% of which were born through ICSI, born in 1995 and 1996. Detailed information on the congenital malformations was collected through specific questionnaires addressed to both the mothers of the IVF children and obstetricians involved in pregnancy and delivery care. The questionnaires were completed within “2 months after birth. Because no unique identification number is available in the Netherlands, a statistical matching procedure was applied that searched for the following variables: birth date of the child and mother, gender of the birth child, birth order for multiple births, birth weight, and gestational age. Using this matching technique, 89% of the children for the IVF cohort were found (n = 1716). In 79% of the traced records, the conception method was correctly coded as “IVF” and “furthermore, all other birth records with the code coding “IVF” as a conception method (n = 2508) were added to the IVF study population. Therefore, the total study population consisted of 4224 children. Possible differences in registration and classification of congenital malformations were investigated through the National Perinatal Database.

Congenital Malformations were observed in 137 IVF children (3.2%) and 8526 of the NC control group children (2.7%). The overall OR for the risk of any malformation for IVF children compared with NC control group children was 1.20 (95% CI: 1.10-1.33). Similar ORs were found for children with major, minor, and unspecified congenital malformations. For IVFs, there were, however, no longer significant due to the smaller number of congenital malformations in these different subcategories. After correction the confounding factors of “maternal age,” “parity,” and “ethnicity,” the OR was 1.03 (95% CI: 0.86-1.23). Further investigation of congenital malformations occurring in the different organ systems was performed (Figure 1). Except for “skin and abdominal wall malformations” and “chromosomal and syndromal malformations,” the ORs for IVF were slightly higher for every specific organ system (Table 1). The difference only reached statistical significance for cardiovascular malformations (OR=1.56, 95% CI: 1.10-2.12). Further exploration of the cardiovascular system malformations showed that all specific cardiovascular malformations were more frequently reported in the IVF study group, with the ORs ranging from 1.32-1.38.

Results and Interpretation

Congenital malformations are coded by organ system: there are eight different organ systems distinguished with 51 specified and 20 unspecified categories of congenital malformations (Figure 1). This classification was also used with the questionnaires to code the malformations reported. All specific congenital malformations, the total number of congenital malformations per organ system, and the overall incidence of all congenital malformations were compared for the IVF study population and control population. In this study, a distinction was also made between major and minor congenital malformations based on the severity of the malformation. Calculated differences in malformation rates were expressed using odds ratios (OR) and 95% confidence intervals (CI). The x2 (Chi Squared Test) was used to test for any significant differences in the malformation rate (P < 0.05). The Fischer Exact Test was used very small and the logistic regression model was used to correct the estimated OR for the overall number of malformations for distribution of maternal characteristics “age of mother,” “parity,” and “ethnicity” by introducing them into the model as covariates. Statistical analyses were performed in Statistical Package for the Social Sciences (SPSS) version 10.

Results and Interpretation (contd.)

However, only differences in occurrence of “single umbilical artery” reached statistical significance (OR=1.93, 95% CI:1.11-3.35). Neural tube defects did not occur more frequently in the IVF study group. Although the ORs of all specific cardiovascular malformations were not statistically significant, the malformations that occurred significantly more frequently in IVF were relatively minor: “single umbilical artery,” “inguinal hernia,” “club foot,” and “other unspecified skeletal and muscular malformations.” These findings could, however, be misleading, as the findings due to birth defects are not covered by insurance, parents may be burdened with the costs of medical care. With studies and research, health issues and possible financial problems due to IVF and ICSI can be significantly reduced, if not eliminated. Parents will be more hopeful their children can be born without congenital malformations.

Relevant Applications to Biotechnology

Advancements in biotechnology have allowed Assisted Reproductive Technology (ART) to grow and become an option for people with infertility and other health issues. ART continues to become more precise and a wider variety of options within ART are available. Research will be conducted to improve the lives of the children affected by the causes of their malformations and chances of congenital malformations will be lowered and eventually eradicated.

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References

The effects of Letrozole and Clomiphene Citrate on ligands expression of Wnt3, Wnt7a, and Wnt8b in proliferative endometrium in women with PCOS. The graph demonstrates the effects of LE and CC on the expression of (A) LIF; (B) FGF-22; (C) DKK-1; and (D) MMP-10 mRNA expression in the endometrium. Overall, gene expression is higher in LE group which results in a healthier and thicker endometrium.

Table 2 demonstrates the effects of Letrozole versus Clomiphene Citrate therapy for PCOS women in the intention-to-treat analysis. The patients had significantly higher pregnancy, ovulation, and live birth rates.

The graph demonstrates the effects of LE and CC on the expression of (A) LIF; (B) FGF-22; (C) DKK-1; and (D) MMP-10 mRNA expression in the endometrium. Overall, gene expression is higher in LE group which results in a healthier and thicker endometrium.

Mobile ovarian syndrome is one of the most common causes of female infertility that affects about 5% of women. They are treated with 2.5 mg of LE. Treatment was administered orally for 5 days beginning on day 3 until live birth (LR) rates and CC were 48.8% and 35.4%, respectively. Although there was not a major difference in LB rates (p=0.089), a higher expression of proteins responsible for endometrial proliferation during the luteal phase and higher progesterone levels during CC-treated women. Letrozole increased LIF (immunostained in glandular epithelial and endometrial stromal cells) expression in comparison to the women treated with CC (p=0.007). However, there was no significant difference in the number of miscarriages, anomalies, multiple or loss in pregnancies. Therefore, the patients’ and their partner’s fertility were monitored for the immunoexpression of gene markers important for implantation: LIF (leukemia inhibitory factor). A low mRNA or protein expression of LIF is correlated to infertility; therefore, LE can be used as a predictor for implantation, subsequent pregnancy and live birth. If ovulation was not achieved, the dose was increased. Patients were monitored with ultrasound follicle detection test to determine ovulation and performed vaginal ultrasound biopsies 7 days later. Blood was also collected for estradiol and progesterone hormone levels. The endometrial thickness of proteins--PCR is used for amplifying a segment of DNA with gene primers shown to be regulated differently based on the agent--after the biopsy. Lastly, mRNA analysis and the pipette suction curettes, for performing endometrial biopsies, are necessary to determine the genes expressed with LE and CC therapy.

LE was randomly given either 50 mg of CC (75) or 2.5 mg of letrozole (75) until pregnancy or for up to 6 oocytes cycles. Preserving mono-ovulation. In the study deduced that the lower expression of proteins responsible for endometrial proliferation, resulting in a higher percent of CC-women having a thin endometrium. Polycystic ovarian syndrome is one of the most common causes of female infertility that affects about 5% of women. They are treated with 2.5 mg of LE. Treatment was administered orally for 5 days beginning on day 3 until live birth (LR) rates and CC were 48.8% and 35.4%, respectively. Although there was not a major difference in LB rates (p=0.089), a higher expression of proteins responsible for endometrial proliferation during the luteal phase and higher progesterone levels during CC-treated women. Letrozole increased LIF (immunostained in glandular epithelial and endometrial stromal cells) expression in comparison to the women treated with CC (p=0.007). However, there was no significant difference in the number of miscarriages, anomalies, multiple or loss in pregnancies. Therefore, the patients’ and their partner’s fertility were monitored for the immunoexpression of gene markers important for implantation: LIF (leukemia inhibitory factor). A low mRNA or protein expression of LIF is correlated to infertility; therefore, LE can be used as a predictor for implantation, subsequent pregnancy and live birth. If ovulation was not achieved, the dose was increased. Patients were monitored with ultrasound follicle detection test to determine ovulation and performed vaginal ultrasound biopsies 7 days later. Blood was also collected for estradiol and progesterone hormone levels. The endometrial thickness of proteins--PCR is used for amplifying a segment of DNA with gene primers shown to be regulated differently based on the agent--after the biopsy. Lastly, mRNA analysis and the pipette suction curettes, for performing endometrial biopsies, are necessary to determine the genes expressed with LE and CC therapy.

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