POSTER PRESENTATION BASICS

Vivian Puusepp
Department of Philosophy, University of Tartu

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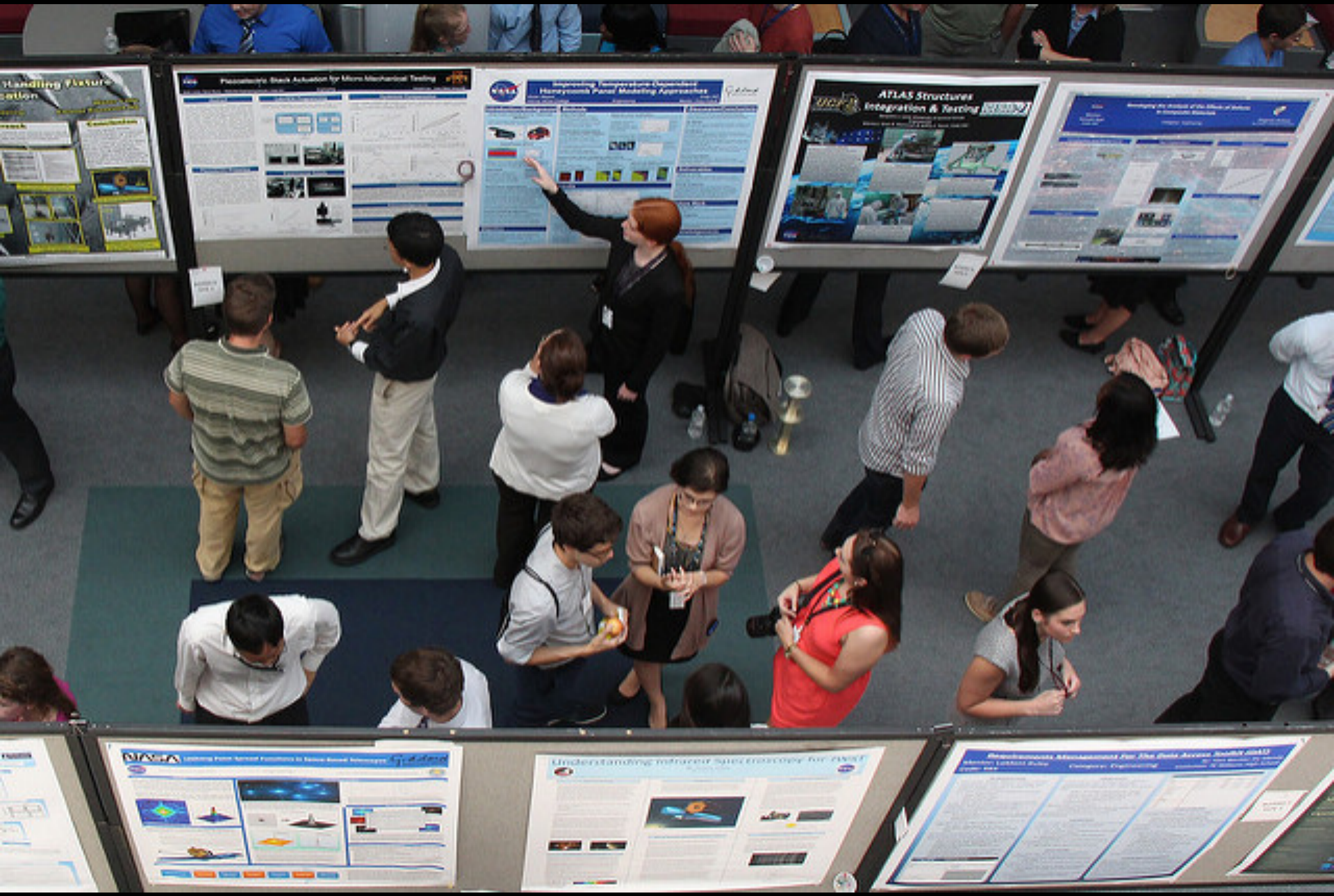

Piezoelectric Stack Actuation for Micro Mechanical Testing
Improving Temperature Dependent Honeycomb Panel Modeling Approaches
ATLAS Structures Integration & Testing
Understanding Infrared Spectroscopy for PWST

POSTER PRESENTATIONS

- Widely spread in the academic world.
  - Very common in the natural and social sciences.
  - Gaining more popularity also in the humanities.
- Enables you to present your research in an attractive and condensed form.
- Visual language: tables, graphs, pictures.

POSTER SESSIONS

Presenters stand by their posters, ready to introduce their research to the interested participants. This includes:

- "walking through" the poster with the participants;
- receiving feedback;
- answering questions;
- making new contacts.

POSTER SESSIONS

- A wonderful opportunity to:
  - introduce yourself and your research to the academic community;
  - receive useful feedback;
  - make new contacts.

- Highly competitive:
  - your poster should catch attention and forward your message in a fast, clear and apposite manner.

Going to a conference

- Choose a suitable conference.
- Find out what you need to do in order to apply for a conference.
- Make sure you know the deadlines.
  - Some conferences accept only ready made posters/papers, others accept abstracts.

What makes a good poster?

- Short and catchy title.
- Word count between 300 and 800 words.
- Clear, concise and apposite text – less is more!
- Text visually structured for an easy read (subtitles, numbering, bullet points etc).
- Skillful use of fonts, colors and graphics.
- Well-balanced design.

How to start?

Ask yourself:

- What is the most important and interesting finding or result of my current research?
- How can I share this result or finding with others using visual means?
  - How could I use tables, graphs, photos, drawings etc to make my point?
- What information can I transmit verbally to complement the information on the poster?

What software to use?

- PowerPoint: popular, easy to use.
- Adobe Illustrator, Photoshop, InDesign: professional, requires more skills, more expensive.
- OpenOffice, Inkscape, Gimp, Gliffy, Lovely Charts etc: free software.

Designing your poster

- Start early! Making a good poster takes time.
  - You also need extra time for printing the poster. Make sure you know where and when you can print it and how to pay for it.
- Make sure you know what is the required format for the posters (size, landscape/portrait).
- Know your audience and address your message to the particular target group!

Designing your poster

- What is the one thing that participants should remember from your poster presentation?
- Important information must be readable from a 3-4 meters distance.
- Different parts of the poster should be logically ordered.

Designing your poster

- Use simple and apposite illustrations. Make your text short and clear. The main point of your poster should be graspabale within 30 seconds!
- Do not exaggerate with colors and fonts! 40% of the surface of the poster should be empty space. Less is more!
- The poster should speak for itself – i.e. it should make sense without an additional explanation.

Poster components:

- Title.
- Information about the author(s) and their affiliation, contact information.
- Abstract (optional).
- Clearly stated research question or problem.
- Description of the methods (in case of empirical research).
- Results or conclusion.
- Most important references.
- Logos of the sponsors.

Logo

Title of the Research Study

PEOPLE WHO DID THE STUDY

UNIVERSITIES AND/OR HOSPITALS THEY ARE AFFILIATED WITH

Logo

Introduction

We hope you find this template useful! This one is set up to yield a 80x90 centimeter vertical poster.

We've put in the headings we usually see in these posters, you can copy and paste and change to your hearts content! We suggest you use keep black text against a light background so that it is easy to read. Background color can be changed in format-background-drop down menu.

The boxes around the text will automatically fit the text you type, and if you click on the text, you can use the little handles that appear to stretch or squeeze the text boxes to whatever size you want. If you need just a little more room for your type, go to format-line spacing and reduce it to 90 or even 85%.

The dotted lines through the center of the piece will not print, they are for alignment. You can move them around by clicking and holding them, and a little box will tell you where they are on the page. Use them to get your pictures or text boxes aligned together.

How to bring things in from Excel® and Word®

Excel- select the chart, hit edit-copy, and then edit-paste into PowerPoint®. The chart can then be stretched to fit as required. If you need to edit parts of the chart, it can be ungrouped. **Watch out** for scientific symbols used in imported charts, which PowerPoint will not recognize as a used font and may print improperly if we don't have the font installed on our system. It is best to use the Symbol font for scientific characters.

Word- select the text to be brought into PowerPoint, hit edit-copy, then edit-paste the text into a new or existing text block. This text is editable. You can change the size, color, etc. in format-text. We suggest you not put shadows on smaller text. Stick with Arial and Times New Roman fonts so your collaborators will have them.

Scans

We need images to be 72 to 100 dpi in their final size, or use a rule of thumb of 2 to 4 megabytes of uncompressed .tif file per square foot of image. For instance, a 3x5 photo that will be 6x10 in size on the final poster should be scanned at 200 dpi.

We prefer that you import tif or jpg images into PowerPoint. Generally, if you double click on an image to open it in Microsoft Photo Editor, and it tells you the image is too large, then it is too large for PowerPoint to handle too. We find that images 1200x1600 pixels or smaller work very well. Very large images may show on your screen but PowerPoint cannot print them.

Preview: To see your in poster in actual size, go to view-zoom-100%. Posters to be printed at 200% need to be viewed at 200%.

Feedback: If you have comments about how this template worked for you, email to sales@megaprint.com.

We listen! Call us at 800-590-7850 if we can help in any way.

Methods

Xxxx

Interactions between oxytocin and dopamine underlie perspective-taking: a genetic study

Irene Trilla[1], Anne Weigand[1], Yan Fan[2], Malek Bajbouj[2] and Isabel Dziobek[1]

[1]Berlin School of Mind and Brain and Institute of Psychology, Humboldt-Universität zu Berlin, Berlin, Germany
[2]Department of Psychiatry, Campus Benjamin Franklin, Charité, Berlin, Germany

irene.trilla@hu-berlin.de

Background

While a role of the oxytocin system in empathic behavior has been well established, its neurobiological mechanisms are yet not fully understood. As one explanation, oxytocin might interact with the dopaminergic reward system to increase sensitivity to social rewards, which would in turn enhance social motivation and facilitate empathy (1). In line with this hypothesis, the oxytocin receptor (OXTR) gene has been shown to predict dopaminergic functioning (2), and interactions between the oxytocin- and dopamine-related genes have been associated with amygdala reactivity to social stimuli (3). In this study, we are examining the interplay between oxytocin- and dopamine-related gene variants underlying empathy.

We predicted that:

1) The OXTR rs53576 polymorphism would be associated with individual differences in empathy.
2) The effects of OXTR rs53576 would be modulated by the ANKK1 TaqIA polymorphism.

OXTR gene

Function: encoding of the oxytocin receptor

SNP: rs53576

Alleles: A < G

A allele linked to:
- Lower social cognition
- Autism spectrum disorders

ANKK1 gene

Function: control of dopamine synthesis

SNP: TaqIA

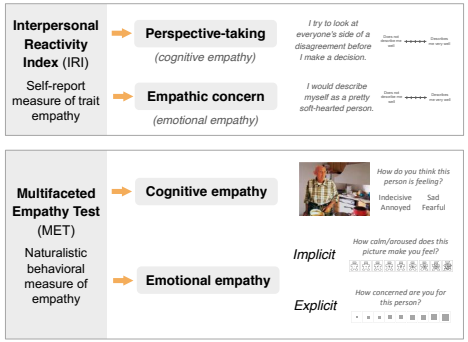
Alleles: A1 < A2

A1 allele linked to:
- Lower reward sensitivity
- Lower striatal D2 receptor

Methods

Sample: 358 healthy individuals (56% females; M_{age} = 41.36, SD_{age} = 17.63, $range_{age}$ = 18-77 years)

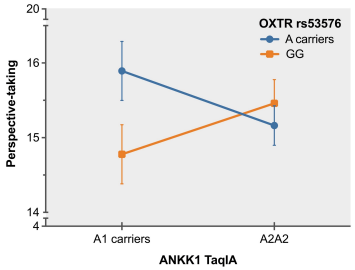
Multidimensional empathy assessment:



Genotyping: DNA was extracted from whole blood and SNPs were genotyped using iPLEX®Reagents MassARRAY®. A-allele carriers (AA + AG) for the OXTR rs53576, and A1-allele carriers (A1A1 + A1A2) for the ANKK1 TaqIA were treated as one group, respectively. All genotype distributions were in Hardy-Weinberg equilibrium.

Results

1) No sig. main effect of OXTR genotype on any IRI or MET subscales
2) OXTR - ANKK1 interaction on IRI Perspective-taking (age as covariate): $F(1, 40.438) = 4.302$, $p = 0.039$, $\eta_p^2 = 0.012$



Discussion

- In contrast to previous studies (e.g., 4), OXTR rs53576 was not associated with individual differences in empathy in our sample.
- Gene-by-gene interactions were found for perspective-taking, but not for any other trait or behavioral measures of empathy. Given the absent correlations between the cognitive empathy subscales of the IRI and MET ($r = 0.041$, $p = 0.442$), the interaction effect between OXTR rs53576 and ANKK1 TaqIA genotypes seems to be selective for specific facets of cognitive empathy.

Conclusion: Albeit preliminary, our results hint at an interplay between the oxytocin and dopaminergic reward systems in shaping empathic abilities. Notably, the effects seem to be confined to perspective-taking, which suggests the existence of different neurobiological mechanisms underlying cognitive and emotional components of empathy.

References

(1) Bethlehem RAI, Baron-Cohen S, van Honk J, Auyeung B, Bos PA (2014) The oxytocin paradox. *Front Behav Neurosci* 8:48
(2) Love T, Enoch M-A, Hodgkinson CA, Peciña M, Mickey BJ, Koeppe RA, Stohler CS, Goldman D, Zubieta JK (2012) Oxytocin Gene Polymorphisms Influence Human Dopaminergic Function in a Sex Dependent Manner Tiffany. *Biol Psychiatry* 72:198–206
(3) Sauer C, Montag C, Reuter M, Kirsch P (2013) Imaging oxytocin × dopamine interactions: an epistasis effect of CD38 and COMT gene variants influences the impact of oxytocin on amygdala activation to social stimuli. *Front Neurosci* 7:45
(4) Uzefovsky F, Shalev I, Israel S, Edelman S, Raz Y, Mankuta D, … Ebstein RP (2015). Oxytocin receptor and vasopressin receptor 1a genes are respectively associated with emotional and cognitive empathy. *Horm Behav* 67:60–65.

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Multicultural Motherhood: Seabrook Farms in the 1940s & 1950s

Kim LeMoon, Aresty Research Assistant to Mary K. Trigg Ph.D.

RUTGERS
Aresty Research Center for Undergraduates

1944. Charles Seabrook began recruiting thousands of Japanese-Americans from internment camps to live and work at Seabrook Farms. He knew he would have to provide child care if he expected parents to work long hours. By 1950, over a hundred youngsters were enrolled at the Seabrook Child Care Center. They attended Monday through Saturday from 7:30am until 6:00pm and received a hot lunch, an inexhaustible supply of milk, fruit juice, and snacks, and a daily dose of cod liver oil.

1949–1950. More than 650 Estonian refugees who had been staying in displaced persons camps in Germany came to Seabrook Farms with hopes for a new beginning. They were welcomed warmly by the Japanese-Americans at Seabrook, who identified with their plight. Like the Japanese-American mothers had done, the Estonian mothers began a weekend school at the Seabrook Community House to teach language, history, and geography, as well as Estonian songs.

Thanksgiving 1950. Part blizzard, part hurricane, the "Storm of the Century" killed 300 people. In southern NJ, the Delaware Bay flooded, forcing hundreds to evacuate their homes. The Red Cross sent evacuees to the Seabrook Community House, where the Seabrook families did more than just accommodate them. The adults cooked and served food, and distributed dry clothing. The children shared their toys with the young flood victims. They knew first-hand what it felt like to lose one's home.

"One of the lasting memories of my mother is seeing her come over the hill to the nursery school to take me home. Mama, in her blue uniform, coming over the slope after working overtime at the frozen-food factory . . . I would be the last child to be picked up and used to sit at the window and wait. What a safe feeling it was to see my mother finally coming."

Emiko Noguchi – Seabrook resident 1945-1961

About Seabrook Farms

In the 1940s and 1950s, Seabrook Farms in Cumberland County, New Jersey was the largest processor of frozen food in the world. Workers came from over 25 countries and spoke over 30 languages. This included more than 2,500 Japanese-American residents, the highest concentration of this demographic in the United States at that time.

"Looking back, I see Seabrook as a playing field of sorts on which we acquired an outlook on life that makes it easier today to live and work and feel at home in a multicultural America. "Multiculturalism," a popular buzz word . . . is not a new concept. In Seabrook more than forty years ago, it was already a fact of everyday life."

Liina Keerdoja (1994) – Seabrook resident 1949-1960

Preserving Heritage. Mothers at Seabrook created and ran organizations, social clubs, houses of worship, and held special ethnic events to preserve traditions among their children. Festivals and holidays were especially important and Seabrook mothers made these events as inclusive as possible. For example, Estonian mothers were invited to display their beautifully hand-crafted scarves, shawls, sweaters, and dolls at the traditional Japanese Doll Day Festival.

Community House Cafeteria. Open 24/7, free meals were served to families for months after their arrival, until they were able to provide for themselves. Even after that, mothers often worked too much to be able to cook full meals on a regular basis. Both parents worked long hours, one on day shift, the other on night shift, and the cafeteria offered healthy, affordable meals. When they did cook, mothers tried to serve traditional fare so that their children would remember that aspect of their cultural heritage.

Seabrook Farms Traditions. The annual charity Chow Mein Dinner was sponsored by the Japanese-American Citizens League, but the entire Seabrook community came together to make it happen. From Seabrook's managers to the field workers, they decorated, planned entertainment, and cooked and served food to the Upper Deerfield Township patrons who attended. It was during these community-wide events that mothers from dissimilar class and ethnic backgrounds forged lasting friendships.

"Though I was young I was constantly aware of the hardships our parents endured. The long miserable hot hours at the factory and the little sleep they got. I was silently relieved whenever my mother got home early so that she would get rest but not realizing the financial hardships this would cause because of the short days . . . I remember us children standing on the sidewalk watching the foot traffic coming from the factory to see our mother finally appear."

Reet Sikkemae – Seabrook resident 1952-1961

BACKGROUND

The research on motherhood at Seabrook Farms in the 1940s and 1950s was conducted for a book project led by Mary K. Trigg, director of Leadership Programs and Research at the Institute for Women's Leadership, and Associate Professor in the Department of Women's and Gender Studies at Rutgers. Dr. Trigg is interested in looking at representations of motherhood in the U.S. from 1920-1960 from a temporal perspective.

In the fall of 2012, when this project began, the first goal was to find out what had already been published on the topic and, consequently, what had yet to be studied. A dearth of material on immigrant mothers, lesbian mothers, and poor mothers of all stripes was identified.

METHODS

Finding primary sources for the project proved challenging. Kayo Denda, Women's Studies Librarian at the Mabel Smith Douglass Library, recommended looking on the NJ Digital Highway website, a public portal containing archives from cultural heritage institutions in NJ. In particular, she thought the materials from the Seabrook Educational and Cultural Center might be useful for the project.

From the Seabrook collection, 2750 photos from the Postwar period (1945-1970) were studied to understand motherhood at Seabrook Farms. Additional textual sources included "I remember Seabrook" essays written in the 1990s, a masters thesis, and journal and newspaper articles.

DISCUSSION

At Seabrook Farms, a communal type of motherhood existed. Trusted, caring adults supervised the children while mothers labored for long hours at the vegetable factory. Work at Seabrook was arduous, but it facilitated recovery from the economic, political, and social devastation that had befallen these families as a result of WWII.

Despite the challenges, mothers did their best to keep traditions alive while fostering an environment that promoted multicultural learning and acceptance of racial and religious differences. Elucidating the role of mothers in this historically unique community revealed a temporal anomaly divergent from the stereotypes of mothers in the U.S. during this time.

http://www.pet-land.info/poster-presentation-template-humanities/

Visuals

- Have you taken photos you could use?
  - NB! Do not steal pictures from the Internet!
    - https://pixabay.com etc
  - Visuals should support the main message.
  - Make sure the resolution is sufficient for printing!
  - People like pictures of people! ☺
- Could tables or graphs help you explain your results?
  - Tables and graphs should be clear, simple, salient!

Before the event

- Think about how to transport the poster. Paper posters are easy to carry in a special poster tube.

Before the event

- Make sure you know where to print out you poster and how much it costs.
  - In Tartu, posters can be printed out at the UT Multimedia Center, both on paper and on textile. Contact: meedia@ut.ee
  - Ask your department for financial support!
  - Get feedback (from your supervisor or peers) before you send your poster to print.
- It's useful to also print out A4 handouts for participants to take home.

Before the event

- Make sure you have:
  - Paper and pencil for making notes;
  - Means for putting your poster up (e.g. tape, thumbtacks);
- Prepare a short verbal presentation (5-10 minutes) of your research to walk the participants through your poster.
- Think about what questions your audience may have and how to answer them.

Poster session

- Do not just read out your poster!
- Be active, ask questions!
- Thank everyone who has shown interest for your poster, ask for contact details!

https://www.youtube.com/watch?v=vMSaFUrk-FA

O⁶-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Resensitizes Breast Cancer Cells to Anti-Estrogen Therapy

Joshua Smith[1], George C Bobustuc[1], Rafael Madero-Visbal[1], Jimmie Colon[1], Beth Isley[1], Jonathan Ticku[1], Kalkunte S. Srivenugopal and Santhi Konduri[1]

[1]Cancer Research Institute of M.D Anderson Cancer Center Orlando [2]Texas Tech University Health Sciences Center, Amarillo, TX

MD ANDERSON CANCER CENTER ORLANDO

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Abstract

Endocrine therapies using anti-estrogens are least toxic and very effective for breast cancers, however, tumor resistance to tamoxifen remains a stumbling block for successful therapy. Based on our recent study on the involvement of the DNA repair protein MGMT in pancreatic cancer (Clin Cancer Res. 15, 6087, 2009), here, we investigated whether MGMT overexpression mediates tamoxifen resistance. Specifically, we determined whether administration of MGMT inhibitor [O⁶-benzylguanine (BG)] at a non-toxic dose alone or in combination with the anti-estrogens (tamoxifen/fulvestrant) curtails human tamoxifen resistant breast cancer cell growth. Further, we also determined whether BG sensitizes breast cancers to tamoxifen using tamoxifen resistant cells.

MGMT expression was found to be increased in breast cancer cells relative to normal breast epithelial cells. Also, MGMT levels were significantly higher in tamoxifen resistant MCF-7 compared to the parent cells. Silencing of the ER-α expression using a specific siRNA resulted in augmentation of MGMT mRNA and protein levels by 2 fold. We also observed an inverse correlation between MGMT and p53 levels in breast cancer cell lines; moreover, p53 downregulation was accompanied by increased MGMT expression. Other experiments showed that BG alone or BG in combination with tamoxifen or fulvestrant decreased ER-α expression, whereas tamoxifen alone and fulvestrant alone increased and decreased the same respectively. However, all these treatments increased the $p21^{cip1}$ mRNA and protein expression significantly. BG inhibited tamoxifen resistant breast cancer growth in a dose-dependent manner and it also resensitized resistant breast cancer cells to anti-estrogen therapy (TAM/ICI). These combinations also enhanced the cytochrome C release and the PARP cleavage, indicative of apoptosis. In breast cancer xenografts, BG alone or a combination of BG with tamoxifen or fulvestrant caused significant tumor growth delay and immunohistochemistry revealed that BG inhibited the expression of MGMT, ER- α, ki-67 and increased $p21^{cip1}$ staining. These findings suggest that MGMT inhibition may provide a novel and effective approach for overcoming tamoxifen resistance.

Introduction

Recent advances in breast cancer research have identified key pathways involved in the repair of DNA damage induced by chemotherapeutic agents. The ability of cancer cells to recognize DNA damage and initiate DNA repair is an important mechanism for therapeutic resistance and has a negative impact on therapeutic efficacy. A number of DNA-damaging alkylating agents attack the nucleophilic O⁶ position on guanine, forming mutagenic and highly cytotoxic interstrand DNA crosslinks. The DNA repair enzyme O⁶-alkylguanine DNA alkyltransferase (AGT), encoded by the gene MGMT, repairs alkylation at this site and is responsible for protecting both tumor and normal cells from alkylating agents. MGMT is expressed constitutively in normal cells and tissues. In breast tumors, MGMT gene expression is elevated and levels are up to 4-fold higher than in the normal breast. Interestingly, it has been shown that tamoxifen accelerates proteasomal degradation of MGMT in human cancer cells. In 1991, Pegg, Moschel, and Dolan observed that O⁶ benzylguanine (BG) inhibited AGT and potentiated the cytotoxicity of both chloroethylating agents and methylating agents. In a series of important observations, they fully characterized the interaction between BG and AGT and its therapeutic impact. They showed that BG binds AGT, transferring the benzyl moiety to the active-site cysteine [29]. The reaction is very rapid and more potent than any other previously known AGT inhibitor. BG is not incorporated into DNA in living cells and reacts directly with both cytoplasmic and nuclear AGT. Because BG is a psuedosubstrate for MGMT which results in the covalent transfer of benzyl group to the active site cysteine, the MGMT protein is degraded after each reaction. This stoichiometric reaction mechanism effectively depletes the AGT content in tumors and the associated repair of alkylation damage. BG is currently undergoing clinical trials in various cancers to increase the efficacy of alkylating agents.

Interestingly, several observations suggest an inverse correlation between the levels of MGMT and p53 tumor suppressor proteins where wild-type p53 suppresses transcription of human MGMT expression. Unfortunately, p53 function is often inactivated or suppressed in human cancers; therefore, restoration of wt-p53 activity is essential for the success of some treatments. However, whether or not this is mediated by suppression of MGMT expression has yet to be determined. To date, the cross-talk between MGMT and ER-alpha (and the link to p53 expression) has not been explored in drug (i.e., tamoxifen) resistant breast tumors. The anti-estrogen tamoxifen is the most commonly used treatment for patients with estrogen receptor positive breast cancer. Although many patients benefit from tamoxifen in the adjuvant and metastatic settings, resistance to this endocrine therapeutic agent is an important clinical problem. The primary goal of present study was to investigate the mechanisms of anti-estrogen drug resistance and to design new therapeutic strategies for circumventing this resistance. The results show that MGMT expression is increased in TAM-resistant breast cancers and inhibition of MGMT by BG significantly improves TAM-sensitivity.

Results

Prolonged Treatment of Tamoxifen Increases MGMT Expression: We developed a tamoxifen resistant MCF-7 cell line by using prolonged treatment of tamoxifen on the parental ER-positive breast cancer cell line, MCF-7. Tamoxifen-resistant MCF-7 cells proliferate at rates similar to the parental MCF-7. Prolonged treatment of tamoxifen onto MCF-7 cells increased MGMT expression compared to parental MCF-7 cells by 2 fold (Fig.1).

Knocking Down ERα Enhances MGMT Expression in Tamoxifen Resistant Breast Cancer Cells: It is not known whether ERα and MGMT transcriptionally regulate each other in tamoxifen resistant breast cancer cells. We therefore investigated whether down regulation of ERα has any effect on endogenous MGMT expression in these cells. As expected, downregulation of ERα using specific siRNA significantly reduced ERα protein levels in these cells. Western blot analysis was performed and the results in the left panel (Fig. 2A) shows that silencing of ERα increases MGMT expression in these cells, and interestingly, the results in the right panel (Fig.2B) show increased MGMT mRNA levels were increased as assessed by qRT-PCR. These data suggest that ERα–mediated signaling functions to repress MGMT gene expression in breast cancer cells.

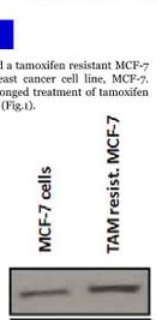


Figure 1. MCF-7 parental and tamoxifen resistant MCF-7 cell pellets were prepared, proteins were isolated and MGMT expression was detected by western blot analysis. Tamoxifen resistant MCF-7 breast cancer cells significantly increased MGMT expression compared to MCF-7 parental cells.

Transcriptional Regulation Between MGMT and p53: Previously, it was reported that p53 negatively regulates MGMT in breast cancer cells. Therefore, we addressed whether or not silencing the p53 enhances endogenous MGMT transcription. Tamoxifen resistant MCF-7 cells were transfected with either p53 siRNA (p53-KD) (Fig.2C) or MGMT siRNA (MGMT-KD) (Fig.2D) along with Non-specific siRNA (NS). MGMT expression was consistently increased in p53 knock down cells, with different experiments showing a – fold augmentation (Fig. 2A) and as expected, knocking down MGMT decreased MGMT transcription where as p53 mRNA levels were unaffected in MGMT knockdown cells (Fig.2D). These results confirm that p53 can regulate MGMT at the transcriptional level.

O⁶-Benzylguanine Plays a Dual Role in Tamoxifen Resistant MCF-7 Cells: Contrasting with the experiments above, next, we studied whether or not knocking down MGMT has any effect on ERα transcription. As expected, knocking down MGMT decreased MGMT gene transcripts. However, it was interesting to find that ERα gene transcription was also reduced after MGMT silencing. (Fig.2E). These data demonstrate that BG has the ability to attenuate the not only the MGMT, but also the ERα transcription, indicating a possible dual role for MGMT blockers in these breast cancer cells.

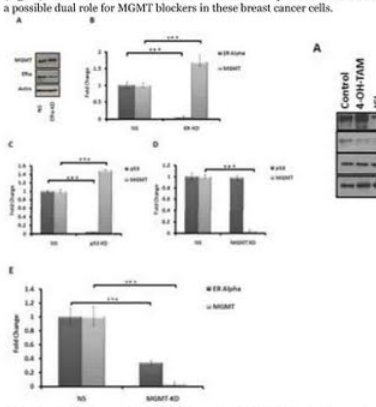


Figure 2. (A) Tamoxifen resistant MCF-7 cells were transfected with ERα siRNA (100nM) (ERα-KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total proteins were isolated and ERα and MGMT expression was determined by western blot analysis. MGMT protein was significantly increased in ERα knock down cells (B) Tamoxifen resistant MCF-7 cells were transfected with ERα siRNA (100nM) (ERα-KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total RNA was isolated and MGMT and ERα transcription was determined by qRT-PCR. MGMT transcription was significantly increased in ERα knock down cells. (C) Total RNA was isolated from non-specific siRNA (NS) (100nM) and p53 siRNA (20 nM) knock down tamoxifen resistant MCF-7 breast cancer cells. MGMT and p53 transcription was determined by qRT-PCR. (D) Total RNA was isolated from non-specific siRNA (NS) (100nM) and MGMT siRNA (100nM) knock down tamoxifen resistant MCF-7 breast cancer cells. MGMT and p53 transcription was determined by qRT-PCR. There is an inverse correlation between MGMT and p53 in tamoxifen resistant breast cancer cells (C & D).

Figure 3. (A) Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μg) and 48h post treatment 4-OH-TAM (1μM), ICI (1μM) either alone or in combination with BG. 24h post treatment cells were harvested and proteins were isolated and western blot analysis was performed. (A) ERα, p53 and MGMT expressions (B) Cytochrome C, PUMA and p21 was determined by western blot analysis (C) tamoxifen resistant MCF-7 cells were treated with or without BG for 48h and later treated with staurosporin (5 μM/L) for 6 hrs PARP cleavage was determined by western blot analysis.

O6-Benzylguanine Modulates p53 Down-Stream Targeted Protein Expressions: Encouraged by the results reported, we investigated the effect of combination therapy on endogenous MGMT, p53, and ERα protein expressions. As expected, BG decreased MGMT expression, while combination therapy (4-OH-TAM or ICI combined with BG) significantly decreased both MGMT and ERα expressions. BG alone or in combination with tamoxifen or ICI decreased ER-α expression, whereas tamoxifen alone and ICI alone increased and decreased the same respectively (Fig.3A). p53 expression was slightly altered after ICI treatment. The reduction in p53 expression by ICI alone was reversed when BG was combined (Fig.3A). We investigated the effect of BG on proteins which are involved in cell cycle regulation, apoptosis in tamoxifen resistant breast cancer cells. All these treatments significantly increased the $p21^{cip1}$ protein expression (Fig.3B). PUMA expression was also increased with these treatments. Hence, PUMA may have translocated to the mitochondria, cytochrome C is released (Fig.3B), and apoptosis was triggered in these cells in presence of combination therapy. PARP cleavage is seen in BG treated cells in presence of staurosporin as an indicative of apoptosis (Fig.3C). Therefore, this data suggest that BG promotes cell cycle arrest and can induce apoptosis by modulating p53 function.

O6-Benzylguanine Modulated Transcriptional Targets in Tamoxifen Resistant Breast Cancer Cells: The effect of combination therapy on endogenous MGMT mRNA levels ws aso stuid. Quantitative real-time PCR (qRT-PCR) resulted that anti-estrogens (TAM/ICI) increased the MGMT expression while the combination therapy decreased it compared to control levels. ERα transcription was decreased compared to controls with all these treatments (Fig.4A). Surprisingly, p21 and PUMA mRNA was significantly increased in the presence of combination treatments (Fig.4B &C). These results suggests that p53 mediated target gene transcription was affected by the drug combinations in breast cancer cells (Fig. 3 & 4).

O6-Benzylguanine Enhances p21 Transcriptional Activity in Tamoxifen Resistant Breast Cancer Cells: In order to investigate the effect of BG on p53 function, we performed luciferase reporter assays. Tamoxifen resistant MCF-7 breast cancer cells were transfected with p21 luc promoter construct in presence or absence of BG (target gene of p53). These results clearly demonstrate that BG significantly enhanced p21 transcriptional activity by 4-5 fold in these cells (Fig.4D).

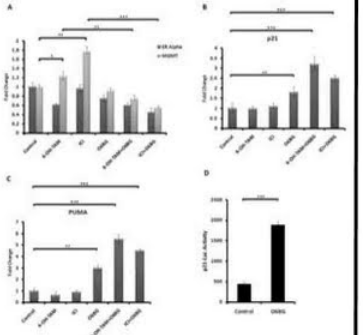
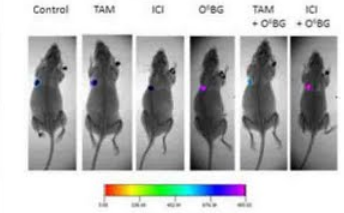


Figure 4. Tamoxifen resistant MCF-7 breast cancer cells were treated in presence or absence of BG (50 μg) for 48h and later 4-OH tamoxifen and ICI (1μM) was either alone or in combination with BG and 24h later cells were harvested and total RNA was isolated. (A) MGMT and ERα (B) p21 transcription (C) PUMA transcription was determined by qRT-PCR. 4-OH tamoxifen and ICI induces MGMT transcription. BG induced PUMA and p21 transcription. (D) Tamoxifen resistant MCF-7 breast cancer cells were transfected with p21-luc construct and 6h later treated with BG and 24h later cells were harvested. p21 transcriptional activity was significantly increased by BG in these cells.

O6-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Increase Resistant Breast Cancer Cell Sensitivity to Anti-Estrogen Therapy (TAM/ICI): Detailed necropsy revealed that all the mice had tumors in the breast. The data summarized in Table 1 show the daily BG alone or in combination with twice weekly tamoxifen/ICI significantly decreased median tumor volume and weight as compared with that seen in tamoxifen/ICI treated and control mice. The combination of BG with tamoxifen or ICI produced the greatest decrease in median tumor volume as compared with control mice (83.99 mm^3, 9.33 mm3 (TAM+BG), respectively; p< 0.0001); (83.99 mm^3, 31.60 mm^3 (ICI+BG), respectively; p<0.0001). Tumor weight was also significantly reduced in mice treated with combination therapy as compared with control mice (81.23 mg, 22.30 mg (TAM+BG), respectively, p<0.0005); (81.23 mg, 51.57 mg (ICI+BG), respectively, p<0.0005). (Table.1). Body weight was not changed among all treatment groups as compared with control mice. No visible liver metastases were present (enumerated with the aid of a dissecting microscope) in all treatment groups.

Histology and IHC Analysis: We next determined the *in vivo* effects of BG (alone or in combination) with tamoxifen/ICI. Tumors harvested from different treatment groups were processed for routine histological and IHC analysis. Tumors from mice treated with BG alone or in combination with tamoxifen/ICI exhibited a significant decrease in MGMT, ERα, ki-67 as compared with tumors treated with tamoxifen/ICI alone or control group. p53 expression was not much altered in these treatment groups. In sharp contrast, the expression of p21 was significantly increased in tumors from mice treated with BG either alone or in combination with tamoxifen/ICI. The images were analyzed by ImageJ (NIH) and MGMT, ERα, p53, p21 and ki-67 expressions were quantified by the ImmunoRatio plugin. (Fig.5).



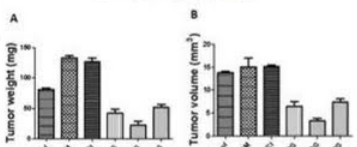
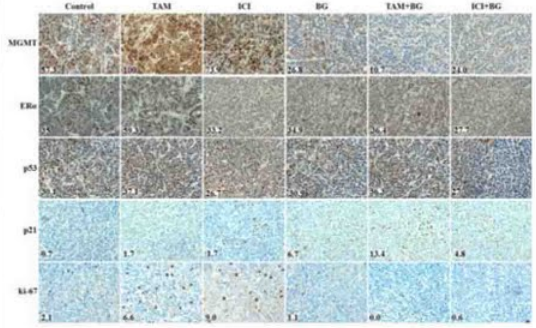


Figure 5. Tumors were harvested from control mice and mice treated with tamoxifen/ICI, BG, or both tamoxifen/ICI and BG. The sections were immunostained for expression of MGMT, ERα, p53, p21 and ki-67. Tumors from mice treated with BG either alone or in combination with tamoxifen or ICI had a significant decrease in the expression of MGMT, ERα and ki-67. p53 expression was not much altered in these treatment groups. In sharp contrast, expression of p21 was significantly increased in all these treatment groups compared to controls. Representative samples (40X) are shown.



Conclusions

1. In the present study, we observed that prolonged treatment with anti-estrogens causes drug resistance by inducing the DNA repair protein O⁶-methylguanine DNA methyltransferase (MGMT).
2. Decreasing the expression of MGMT by exposing breast cancer cells to BG sensitized these cells to anti-estrogen therapy (tamoxifen and ICI 182,780).
3. We also observed that combination therapy of anti-estrogens and MGMT blockers not only overcame the MGMT derived drug (tamoxifen and ICI) resistance but also increased the efficacy of anti-estrogen therapy by decreasing estrogen receptor expression and restoration of the functional activity of p53 in tamoxifen-resistant breast cancer cells.
4. Combination therapy inhibited tamoxifen resistant breast tumor growth *in vivo*.

Acknowledgements

We would like to thank the Florida Department of Health, Bankhead-Coley Cancer Research Program 09BN-20 for their funding of this project.

O⁶-Benzylguanine Inhibits Tamoxifen Resistant Breast Cancer Cell Growth and Resensitizes Breast Cancer Cells to Anti-Estrogen Therapy

Joshua Smith[1], George C Bobustuc[1], Rafael Madero-Visbal[1], Jimmie Colon[1], Beth Isley[1], Jonathan Ticku[1], Kalkunte S. Srivenugopal and Santhi Konduri[1]

[1]Cancer Research Institute of M.D Anderson Cancer Center Orlando [2]Texas Tech University Health Sciences Center, Amarillo, TX

Supported by the Charles Lewis Institute

Posters rarely need abstracts

Text dissolves into intimidating, boring gray

Too small and too much

Caption not aligned with figure

Crammed!

Crammed!

Abstract

Endocrine therapies using anti-estrogens are least toxic and very effective for breast cancers, however, tumor resistance to tamoxifen remains a stumbling block for successful therapy. Based on our recent study on the involvement of the DNA repair protein MGMT in pancreatic cancer (Clin Cancer Res. 15, 6087, 2009), here, we investigated whether MGMT overexpression mediates tamoxifen r… Specifically, we determined whether administration of MGMT inhibitor [O⁶-benzylguanine (BG)] at a non-toxic d… alone or in combination with the anti-estrogens (tamoxifen/fulvestrant) cont… ils human tamoxifen resistant breast cance… cell growth inhibitory effect… breast cancer cells to tamoxifen using tamoxifen resistant cel…

MGMT expression w… found to be increased in breast cancer cells relative to normal breast epithel… al cells. Also, MGMT levels were significant… higher in tamoxifen resistant MCF-7 cells (MCF-7/TAM) than in parental MCF-7 cells. We also observed the ER-α expression using a specific siRNA… resulted in increased MGMT expression. We also observed an inverse correlation between … MGMT and p53 levels in breast cancer cell lines; moreover, p53 downregulation was accompanied by increased MGMT expr… Other experiments showed that BG alone or BG in combination with tamoxifen or fulvestrant decreased ER-α expression, whereas tamoxifen alone and fulvestrant alone increased and decreased the same respectively. However, all these treatments increased the p21^{Cip1} mRNA and protein expression significantly. BG inhibited tamoxifen resistant breast cancer growth in a dose-dependent manner and it also resensitized resistant breast cancer cells to anti-estrogen therapy (TAM/ICI). These combinations also enhanced the cytochrome C release and the PARP cleavage, indicative of apoptosis. In breast cancer xenografts, BG alone or a combination of BG with tamoxifen or fulvestrant caused significant tumor growth delay and immunohistochemistry revealed that BG inhibited the expression of MGMT, ER- α, ki-67 and increased p21^{Cip1} staining. These findings suggest that MGMT inhibition may provide a novel and effective approach for overcoming tamoxifen resistance.

Introduction

Recent advances in breast cancer research have identified key pathways involved in the repair of DNA damage induced by chemotherapeutic agents. The ability of cancer cells to recognize DNA damage and initiate DNA repair is an important mechanism for therapeutic resistance and has a negative impact on therapeutic efficacy. A number of DNA-damaging alkylating agents attack the nucleophilic O⁶ position on guanine, forming mutagenic and highly cytotoxic interstrand DNA crosslinks. The DNA repair enzyme O⁶-alkylguanine DNA alkyltransferase (AGT), encoded by the gene MGMT, repairs alkylation at this site and is responsible for protecting both tumor and normal cells from alkylating agents. MGMT is expressed constitutivel… In normal cells… tissues, however, MGMT gene expression is elevated and levels are up to 4-fold higher than in normal breast tissue. Interestingly, it has been shown that tamoxifen accelerates proteasomal degradation of MGMT… benzylguanine (BG) inhibited AGT and p… ents. In a series of important observation… therapeutic impact. They showed that BG binds AGT, transferring the benzyl moiety to the active-site cysteine [29]. The reaction is very rapid and more potent than any other previously… ing cells and reacts directly with both cyt… MGMT which results in the covalent transfer of benzyl grou… to the active… reaction. This stoichiometric reaction mechanism e… effectively depletes the AGT content in tumors and the associated repair of alkylation damage. BG is currently undergoing c… linical trials in various cancers to increase the efficacy of alkylating agents.

Interestingly, several … of MGMT and p53 tumor suppressor proteins where wild-t… p53 suppresses transcription of human MGMT expression. Unfortunately, p53 function is often inactivated or suppres… in human cancers; therefore, restoration of wt-p53 activity is essential for the success of some treatments. However, whether or not this is mediated by suppression of MGMT expression has yet to be determined. To date, the cross-talk between MGMT and ER-alpha (and the link to p53 expression) has not been explored in drug (i.e., tamoxifen) resistant breast tumors. The anti-estrogen tamoxifen is the most commonly used treatment for patients with estrogen receptor positive breast cancer. Although many patients benefit from tamoxifen in the adjuvant and metastatic settings, resistance to this endocrine therapeutic agent is an important clinical problem. The primary goal of present study was to investigate the mechanisms of anti-estrogen drug resistance and to design new therapeutic strategies for circumventing this resistance. The results show that MGMT expression is increased in TAM-resistant breast cancers and inhibition of MGMT by BG significantly improves TAM-sensitivity.

Results

Prolonged Treatment of Tamoxifen Increases MGMT Expression: We developed a tamoxifen resistant MCF-7 cell line by using prolonged treatment of tamoxifen on the parental ER-positive breast cancer cell line, MCF-7. Tamoxifen-resistant MCF-7 cells proliferate at rates similar to the parental MCF-7. Prolonged treatment of tamoxifen onto MCF-7 cells increased MGMT expression compared to parental MCF-7 cells by 2 fold (Fig.1).

Knocking Down ERα Enhances MGMT Expression in Tamoxifen Resistant Breast Cancer Cells: It is not known whether ERα and MGMT transcriptionally regulate each other in tamoxifen resistant breast cancer cells. We therefore investigated whether down regulation of ERα has any effect on endogenous MGMT expression in these cells. As expected, downregulation of ERα using specific siRNA significantly reduced ERα protein levels in these cells. Western blot analysis was performed and the results in the left panel (Fig. 2A) shows that silencing of ERα increases MGMT expression in these cells, and interestingly, the results in the right panel (Fig.2B) show increased MGMT mRNA levels were increased as assessed by qRT-PCR. These data suggest that ERα-mediated signaling functions to repress MGMT gene expression in breast cancer cells.

Transcriptional Regulation Between MGMT and p53: Previously, it was reported that p53 negatively regulates MGMT in breast cancer cells. Therefore, we addressed whether or not silencing the p53 enhances endogenous MGMT transcription. Tamoxifen resistant MCF-7 cells were transfected with either p53 siRNA (p53-KD) (Fig.2C) or MGMT siRNA (MGMT-KD) (Fig.2D) along with Non-specific siRNA (NS). MGMT expression was consistently increased in p53 knock down cells, with different experiments showing a ~ fold augmentation (Fig. 2A) and as expected, knocking down MGMT decreased MGMT transcription where as p53 mRNA levels were unaffected in MGMT knockdown cells (Fig.2D). These results confirm that p53 can regulate MGMT at the transcriptional level.

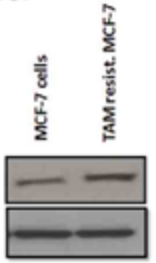


Figure 1. MCF-7 parental and tamoxifen resistant MCF-7 cell pellets were prepared, proteins were isolated and MGMT expression was detected by western blot analysis. Tamoxifen resistant MCF-7 breast cancer cells significantly increased MGMT expression compared to MCF-7 parental cells.

O⁶-Benzylguanine Plays a Dual Role in Tamoxifen Resistant MCF-7 Cells: Contrasting with the experiments above, next, we studied whether or not knocking down MGMT has any effect on ERα transcription. As expected, knocking down MGMT decreased MGMT gene transcripts. However, it was interesting to find that ERα gene transcription was also reduced after MGMT silencing. (Fig.2E). These data demonstrate that BG has the ability to attenuate the not only the MGMT, but also the ERα transcription, indicating a possible dual role for MGMT blockers in these breast cancer cells.

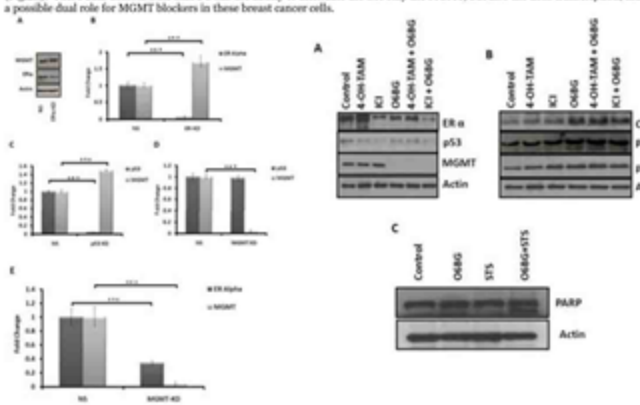


Figure 2. (A) Tamoxifen resistant MCF-7 cells were transfected with ERα siRNA (100nM) (B… ERα KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total … was isolated and MGMT and ERα transcripts were determined … by qRT-PCR. (C) Tamoxifen resistant MCF-7 cells were transfected with p53 siRNA (100 nM) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total RNA was isolated and MGMT and p53 transcripts were determined by qRT-PCR. (D) Tamoxifen resistant MCF-7 cells were transfected with MGMT siRNA (100nM) (MGMT-KD) and NS siRNA (100nM) (NS), and cells were harvested 72h post transfection. Total RNA was isolated and MGMT and ERα transcripts were determined by qRT-PCR. MGMT transcription was significantly increased in ERα knock down cells. (C) Total RNA was isolated from non-specific siRNA (NS) (100nM) and p53 siRNA (100 nM) knock down tamoxifen resistant MCF-7 breast cancer cells. MGMT and p53 transcription was determined by qRT-PCR. (D) Total RNA was isolated from non-specific siRNA (NS) (100nM) and MGMT siRNA (100nM) knock down tamoxifen resistant MCF-7 breast cancer cells. MGMT and p53 transcription was determined by qRT-PCR. There is an inverse correlation between MGMT and p53 in tamoxifen resistant breast cancer cells (C & D).

O6-Benzylguanine Modulates p53 Down-Stream Targeted Protein Expressions: Encouraged by the results reported, we investigated the effect of combination therapy on endogenous MGMT, p53, and ERα protein expressions. As expected, BG decreased MGMT expression, while combination therapy (4-OH-TAM or ICI combined with BG) significantly decreased both MGMT and ERα expressions. BG alone or in combination with tamoxifen or ICI decreased ER-α expression, whereas tamoxifen alone and ICI alone increased and decreased the same respectively (Fig.3A). p53 expression was slightly altered after ICI treatment. The reduction in p53 expression by ICI alone was reversed when BG was combined (Fig.3A). We investigated the effect of BG on proteins which are involved in cell cycle regulation, apoptosis in tamoxifen resistant breast cancer cells. All these treatments significantly increased the p21^{Cip1} protein expression (Fig.3B). PUMA expression was also increased with these treatments. Hence, PUMA may have translocated to the mitochondria, cytochrome C is released (Fig.3B), and apoptosis was triggered in these cells in presence of combination therapy. PARP cleavage is seen in BG treated cells in presence of staurosporin as an indicative of apoptosis (Fig.3C). Therefore, this data suggest that BG promotes cell cycle arrest and can induce apoptosis by modulating p53 function.

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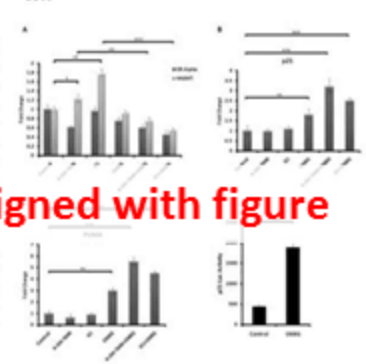

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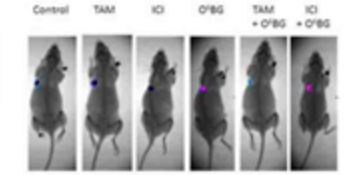

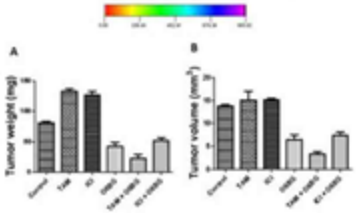

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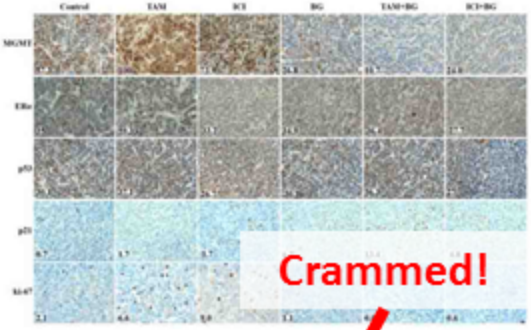

Conclusions

1. In the present study, we observed that prolonged treatment with anti-estrogens causes drug resistance by inducing the DNA repair protein O⁶-methylguanine DNA methyltransferase (MGMT).
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Acknowledgements

We would like to thank the Florida Department of Health, Bankhead-Coley Cancer Research Program 09M01-10 for their funding of this project.

UNIVERSITY OF TARTU
1632

Vivian Bohl

No joint ownership! Shared emotions are social-relational emotions

1. Joel Krueger's Joint Ownership Thesis (JOT)

Sharing the same *token* of a mental state:
"two subjects (infant and caregiver) can be said to simultaneously share the same episode of emotion" (Krueger 2013, 511)

The case of positive emotions in young infants:
- Due to their exogeneous attention young infants experience positive emotions only with the help of caregivers.
- Lack of self-other differentiation in infants' emotional experiences.

2. Critique of JOT

1. Joint ownership or joint subjects?

"the structure of some early infant-caregiver dyadic exchanges is best described as involving joint subjects" (Krueger 2013, 509)

2. *Lumping together phenomenological and ontological claims.*

Phenomenological analysis does not imply ontological claims:
- If infants and caregivers have structurally different experiences (as Krueger argues), they cannot jointly own an emotion.
- Even if the experiences of infants and caregivers have the same structure, it is only necessary, but not sufficient for JOT.

3. The coupling-constitution fallacy.

"(t)he emotion of the caregiver is (…) a constituent part of the infant's emotional experience" (Krueger 2013, 521)
- Even if the emotion of the caregiver is coupled to infant's emotion, it does not follow that the emotion of the caregiver is a constitutive part of infant's emotion.

4. Confusing the extended system that realizes the emotion with the owner of the emotion.
- Even if the extended infant-caregiver system realizes the emotion of the infant, JOT does not follow. Cf. tickling.

3. Emotions and social relationships

Human emotions:
- often arise in social contexts;
- have important social functions;
- can be seen as adaptations for regulating social relationships.

Social-relational emotions arise when subcomponents emotions interact not only intrapersonally, but across two or more people.

Examples:
- Emotional contagion (one-sided);
- Two people yelling at each other, with their level of anger rising in synchrony (two-sided).

4. Making sense of shared emotions

Shared emotions: social-relational emotions that arise in and/or give rise to a *communal relationship.*

Communal relationship – people treat each other as belonging to the same social category by focusing on their commonalities and disregarding distinct individual identities. Involves a sense of oneness with other members of the group.

Example: young infant and caregiver sharing joy

Caregiver's central role	Lack of self-other differentiation
In young infants, positive emotions arise as complements to the positive emotions in caregivers.	Communal relationships focus on a common identity, not on separate individuals.

Reference
Krueger, Joel. 2013. "Merleau-Ponty on shared emotions and the joint ownership thesis." *Continental Philosophy Review*, 46(4): 509–531.

Thank you!

Questions?

vivian.puusepp@ut.ee