Cell cycle delays and chromosome mis-segregation in budding yeast $csm1\Delta$ and $lrs4\Delta$ mutants

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Background Information

• Csm1/Lsr4 is a DNA clamp protein which serves several functions in the budding yeast cell, including:
  – homolog segregation in meiosis I
  – localization of rDNA and telomeres to the nuclear envelope
  – Essential in mono-orientation of kinetochores as part of the monopolin complex.
  – Regulating DNA silencing at rDNA via interactions with RENT complex.

Our goal is to investigate the role of Csm1 and Lrs4 in chromosome segregation in mitosis.
Background Information
Budding Yeast Cell Cycle
Background Information
Cohesin & Cohesion
Research Objectives

Our goal was to investigate the role of Csm1/Lrs4 proteins in mitosis.

- Compare cell cycle progression in wild-type and $csm1\Delta$, $lrs4\Delta$ mutant cells.
- Confirm the sister centromere reassociation defect in nocodazole-treated $csm1\Delta$ and $lrs4\Delta$ mutants.
- To examine the re-establishment of chromosome bi-polar attachment in $csm1\Delta$ and $lrs4\Delta$ mutant cells after nocodazole treatment.
- Perform a Chromatin immunoprecipitation to analyze cohesin binding to centromere in $csm1\Delta$ and $lrs4\Delta$ mutant strains.
Comparing Cell Cycle Progression

Percentage of large budded cells over time in alpha-factor treated cells

- **Percentage of large budded cells**: The graph shows the percentage of large budded cells over time in alpha-factor treated cells. The x-axis represents time in minutes, while the y-axis represents the percentage of large budded cells.

- **Legend**: The graph includes a legend indicating different treatments or conditions, represented by different colored lines.

- **Trends**: The graph illustrates the dynamic changes in the percentage of large budded cells over time, highlighting the impact of the alpha-factor treatment on cell cycle progression.
Confirming the Reassociation Defect
Confirming the Reassociation Defect

Percent cells with 2 GFP signals over time in nocodazole treated synchronized cells

<table>
<thead>
<tr>
<th></th>
<th>90 MINUTES</th>
<th>150 MINUTES</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>wt:csm1</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><strong>wt:lrs4</strong></td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Two-tailed p-values
Synchronous wild type cells 2 hours after nocodazole treatment
Synchronous csm1Δ cells 2 hours after nocodazole treatment
Bi-Polar Re-orientation Assessment
The microtubules (labelled in red) appear to be aligned with the sister chromatids and appear to be in various states of segregation, moving to opposite spindle pole bodies.
These are examples of erroneous microtubule disassociation. In the two images on the left, the spindle is fully elongated but both GFP signals are localized to one of the buds. The two images on the right have spindles that are not running in an axis that would allow for chromosome segregation.
ChIP Analysis

1: non-tagged
2: Lrs4-myc
3: Csm1-myc
4: Nnf1-myc
ChIP Analysis

ChIP Quantification of protein binding to CEN3

binding capacity (scaled)

protein

no tag (negative control)  Lrs4  Csm1  Nnf1 (positive control)

CEN3
Conclusions

- There is a cell cycle delay in $\Delta$Csm1 and $\Delta$Lrs4 mutants in comparison to the wild type strain.

- Csm1 and Lrs4 are involved in sister chromatid interactions.

- Although not as evident as proteins that associate directly with centromeric DNA, the csm1 and lrs4 protein do have some interaction with centromeric DNA based on ChIP analysis.
Acknowledgements

- Dr. Yanchang Wang
- Kelly McKnight
- The Wang Lab (Danny, Fengzhi, Fengshan)
- Dr. Hong-Guo Yu & his lab
- Dr. Ken Brummel-Smith
- Department of Biomedical Science
- The countless number of yeast that I killed over the summer
References

HPV VACCINE ACCEPTANCE IN MIGRANT WORKERS
Introduction

  - HPV vaccine against strains 6, 11, 16, and 18.
  - 3 series within 6 months.

- CDC and FDA recommendations
  - Approved Age 9-26
  - 11-12 year-old girls
  - Before first sexual activity
Why

- 2000 census: Hispanics were the largest and fastest growing minority.
  - Represented 15.4 % of population
  - Projected to be 25% by 2050

- American Cancer Society
  - Hispanic women in the US have twice the incidence of cervical cancer
  - Have 50% higher mortality rate than non-Hispanic Whites

- 1/3 of Hispanic women have not heard about HPV and/or its association with cervical cancer (Bair et al, 2008)
Methodology

- Isabel Collier Read Medical Campus in Immokalee, FL
  - Women’s Health Clinic
  - Pediatrics Clinic

- 200 Hispanic mothers (age ≥ 18) were interviewed

- Brief survey in either English or Spanish
Survey

- Mother’s Gynecological History
- Brief Acculturation Scale
- Social Economic Status
- HPV and HPV Vaccine knowledge and beliefs.
- Daughter’s demographics and HPV vaccination
Participants

- 23% born in the US, 77% not born in the US
  - 74% of non-US born were from Mexico
  - 14% Guatemala
  - Mean length of living in the US: 14 years

- Mean age 36 years old

- Years of education: mean 7 (Sd= 4.4)
  - 10% reported zero years education
  - 20% had graduated from high school
Target daughter

- For mothers with 2 or more eligible daughter, the oldest one was assigned as target daughter.
- Mean age 13 years old (SD=3)
- 95% of daughter were in school
- 27% of daughters had physician recommendation
- 18% had received at least one dose
Results

- Almost half (49%) of the sample had heard of HPV and the HPV vaccine (Gardasil) before taking part in the study
  - Among those who had heard of Gardasil the most common source were TV, doctors, magazines.

- Everyone in the sample (100%) agree that:
  - Vaccines are a good way to protect their children
  - Is important to keep vaccinations up to date

- 90% of moms said they planned to get their daughter vaccinated in the future
Results

- Higher Vaccine uptake in:
  - Daughters whose doctor recommended they get the vaccine (34/35 daughters)
    - 35 vaccinated target daughters
    - 15 had received 1 shot;
    - 9 had received 2 shots
    - 11 had received all 3 shots
  - Older daughters and daughters who had completed more school
  - Moms who had heard of HPV or Gardasil
Results: US acculturation

- US born mothers scored higher on US/American acculturation (correlation = .81) and lower on Latina acculturation (correlation = -.23)
- More likely to have vaccinated their daughters for HPV
- Vaccine attitudes
  - Have concerns about safety of new vaccines
  - Didn’t think their daughters were at risk for HPV
  - Disagree that giving the vaccine would encourage their daughters to have sex
Vaccine attitudes:

- Worry their daughter could get infected with HPV
- Worry about HPV vaccine cost
- If daughter’s pediatrician recommends the HPV vaccine, they would have her vaccinated
  - BUT! only 20% of moms who were born outside of the US received physician recommendations as opposed to 56% of moms born in the US.
Surprising Result: HPV vs. HIV

- **23%** confused HPV and HIV

- All of the 46 women were born outside of the US.
  - majority from Central American countries: Guatemala

- Almost half (47%) of the 34 moms from Central America compared to only 20% of moms from 148 Mexico
Conclusions

- Correlations with higher HPV vaccine uptake
  - Physician recommendation
  - US acculturation
  - Daughter’s age
Recommendations

- Patient education about
  - HPV and HPV vaccine
    - 51% had never heard of either one
    - Exploit media as source: TV and magazines
  - HPV and HIV
    - Central American countries

- Physician recommendations
  - Only 20% of non-US born mothers
Reference

Special Thanks

- Elena Reyes Ph.D.
- Mary Gerend Ph.D.
- George Quesnel M.D.
- Karimu Smith-Barron M.D.
- Javier Rosado Ph.D.
- Jerry Williamson M.D.
Questions?
Recruitment and Retention of Physicians in Rural Areas

- Sarah Weaver -
Rural Health Care Dilemma

- 20% of the US population resides in a rural area
- 9% of physicians practice in a rural area
- Average physician retention is 5-7 years
- 3% of medical school graduates plan on practicing in a rural area
Why?

- High Workload
- Lack of Contact with Colleagues
- Lack of Support from Medical Specialists
- Social Isolation
- Professional Isolation
- Lack of Professional Networks
- Difficulty Finding People to Cover Call
- Family Isolation
- Burn out
My Project

- Questionnaire
- 60-90 Minute Interview
  - Semi-structured interview
  - Improvisational follow-up questions
- Coding/Analysis
  - As individuals first
  - Group consensus
Challenges to Research

- Difficult to find physicians who meet the criteria
  - Practice in a rural area and live in that community
  - Be a primary care specialty
  - Been in the same area for at least 5 years
- Finding physicians willing to participate
- Finding women and minority physicians
Coding

- **Intrinsic**
  - Respect
  - Self-Reliance
  - Persistence
  - Self-Actualization
  - Sense of Place
  - Setting and Achieving Goals

- **Extrinsic**
  - Service
  - Family
  - Variety
  - Familiarity
  - Recreation/Travel
  - Rural lifestyle
  - Income
  - Flexibility
Coding – Rural Background

1 Physician – Rural Upbringing

“Even when I went through medical school, my goal was just to be a doctor, a small town country doctor. I had no idea what my specialty would be. I chose family medicine because it was most closely aligned with what I figured I would be doing in a small town.”

3 Physicians – Positive Rural Experiences

“Well, my grandmother lived in a very small country town and I used to visit her every summer and I just thought it was so neat that we could walk to church, walk to town for a haircut, walk to the bottling plant for Coca Colas and stuff.”
Coding – Community Involvement

“Being involved with the community is very important, particularly when you first start your practice. The more involved you are, the quicker your practice will grow.”
Coding - Variety

“What I enjoy the most is being able to be in 1 room looking after a prenatal patient, in the other room I’m looking at a 2 month old baby, check the next room I’m sewing up a 10 year old who got hit with a golf club and has a skull fracture and I’m putting him back together. Next I’m taking off skin cancer. So I enjoy the variety.”
Conclusion and Applications

- Continued Research
- Purpose of Qualitative Research is to be Hypothesis Generating
- Substantiation of these hypotheses through quantitative research
- Recruitment of students with the Intrinsic characteristics by medical schools
- Incorporating positive rural experiences into medical school curriculums
Special Thanks

- Dr. Manusov, Research Advisor
- Rhonda Collins
- Maribel Mongay
References

Lesbian, gay, bisexual, and transgendered (LGBT) Perceptions on Healthcare in Tallahassee, FL

Jennifer Owen, M2
Jonathan Appelbaum M.D.
Background

- LGBT health disparities exist
- Lack of LGBT-specific medical education
- All physicians will have LGBT patients
- Effective communication with LGBT patients will allow physicians to best care for them
Research Objectives

• Gain a deeper understanding of how patients’ sexual orientation and/or gender identity can influence perceptions and experiences in healthcare – specifically in Tallahassee, FL

• Acquire data to help develop ways to better educate LGBT people on their own health and the role their physicians can play in their life
Methodology

• Questionnaire was developed to elicit the perceptions of the LGBT population in Tallahassee, FL on several areas of healthcare
  – SurveyMonkey.com online survey program

• Participant recruitment
  – Email links sent out through email lists by various LGBT organizations
  – Advertised on Facebook pages for LGBT organizations
  – Email links sent to representatives of LGBT groups at local colleges
  – Flyers posted in “friendly” areas

• The survey was left open through July - August 2010

• Reminder emails sent to maximize participation
Questionnaire Topics

1. Sexuality/gender identity & experiences
2. Experiences with healthcare providers
3. Preferences as a patient
4. Perceptions of Tallahassee, FL
5. Perceptions on history questions
6. Perceptions of health issues
7. Advice to future doctors
### Gender & Sexuality of Participants

<table>
<thead>
<tr>
<th>Participants’ Classification for Gender and Sexual Identity</th>
<th>Lesbian</th>
<th>Gay</th>
<th>Bisexual</th>
<th>Queer</th>
<th>Straight/heterosexual</th>
<th>Other</th>
<th>Total</th>
<th>Percentage of Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Female</td>
<td>30</td>
<td>0</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>36</td>
<td>47%</td>
</tr>
<tr>
<td>Male</td>
<td>1*</td>
<td>26</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>29</td>
<td>38%</td>
</tr>
<tr>
<td>Trans FTM</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1</td>
<td>2</td>
<td>3%</td>
</tr>
<tr>
<td>Trans MTF</td>
<td>0</td>
<td>0</td>
<td>2</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>5</td>
<td>6%</td>
</tr>
<tr>
<td>Intersex</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
<td>1%</td>
</tr>
<tr>
<td>Other 1</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>4</td>
<td>5%</td>
</tr>
<tr>
<td>Total</td>
<td>33</td>
<td>26</td>
<td>11</td>
<td>3</td>
<td>1</td>
<td>3</td>
<td>77</td>
<td>100%</td>
</tr>
</tbody>
</table>

* It is considered that the selection of “lesbian” by a male was done erroneously.
What are the perceptions of Tallahassee, FL in terms of LGBT healthcare?

– Do you think Tallahassee is “friendly” to LGBT population?
  • 49% friendly, 38% neutral, 13% unfriendly

– Are your healthcare needs met by the Tallassee medical community?
  • 64% agree, 15% neutral, 21% disagree

– My doctor addresses my personal medical needs
  • 88% agree, 9% neutral, 3% disagree

– Do you feel that because of your sexuality and/or gender identity that you have to go outside of Tallahassee, FL for medical care?
  • 16% said yes, 84% said no
Preference of Provider’s sexual identity and level of LGBT sensitivity

- I would prefer that my doctor identifies as LGBT.
- I prefer to know that a doctor is LGBT-friendly prior to seeing him/her.
- If my doctor assumed I was heterosexual, I would not return.
- If my doctor was insensitive to my sexuality &/or gender identity, I would not return.
Would they prefer disclosing sexuality &/or gender identity on intake forms?

<table>
<thead>
<tr>
<th>Experience</th>
<th>Yes</th>
<th>No</th>
<th>N/A</th>
</tr>
</thead>
<tbody>
<tr>
<td>My doctor provides space on the intake forms to designate sexuality</td>
<td>12%</td>
<td>70%</td>
<td>18%</td>
</tr>
<tr>
<td>My doctor provides space on the intake forms to designate gender identity</td>
<td>9%</td>
<td>74%</td>
<td>17%</td>
</tr>
<tr>
<td>My doctor asked me about my sexuality when taking my history</td>
<td>25%</td>
<td>69%</td>
<td>6%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Preference</th>
<th>Agree</th>
<th>Neutral</th>
<th>Disagree</th>
</tr>
</thead>
<tbody>
<tr>
<td>I prefer to disclose my sexuality &amp;/or gender identity on an intake form prior to seeing the doctor</td>
<td>39%</td>
<td>36%</td>
<td>25%</td>
</tr>
<tr>
<td>I prefer that my doctor asks me in person about my sexuality &amp;/or gender identity</td>
<td>48%</td>
<td>42%</td>
<td>10%</td>
</tr>
</tbody>
</table>
Do they feel the current basic social history questions are appropriate for eliciting information from LGBT patients?

<table>
<thead>
<tr>
<th>Question</th>
<th>Like</th>
<th>Neutral</th>
<th>Dislike</th>
</tr>
</thead>
<tbody>
<tr>
<td>Are you married?</td>
<td>24%</td>
<td>47%</td>
<td>29%</td>
</tr>
<tr>
<td>What is your living situation?</td>
<td>45%</td>
<td>41%</td>
<td>14%</td>
</tr>
<tr>
<td>Do you have a significant other?</td>
<td>76%</td>
<td>18%</td>
<td>5%</td>
</tr>
<tr>
<td>Do you have a partner?</td>
<td>71%</td>
<td>26%</td>
<td>3%</td>
</tr>
<tr>
<td>Do you have sex with men, women, or both?</td>
<td>51%</td>
<td>30%</td>
<td>19%</td>
</tr>
<tr>
<td>How do you identify your gender?</td>
<td>61%</td>
<td>31%</td>
<td>8%</td>
</tr>
</tbody>
</table>
Suggestions for different questions

• Instead of “Are you married?”:
  – Are you married, in a domestic partnership or long term relationship?
  – Are you in a committed relationship of a long term nature?
  – Do you have any significant relationships which may influence your health or wellbeing?
  – What is your sexual orientation?

• Instead of “Do you have sex with men, women, or both?”
  – How would you describe your sexual orientation? ...coupled with: Are you sexually active?
  – What is your sexual orientation?
  – Do you identify as lesbian/gay, bisexual, or heterosexual? Are you sexually active? With one or multiple partners? Depending on the answer, maybe some questions about safe sex.
  – I would prefer that the question be more about the fact that a person is active sexually than who the person has sex with. If the person is found to have an STD then more thorough questioning is necessary.
Does the LGBT population feel they have different or specific healthcare needs due to their sexual orientation/identity?

- 34% agree
- 31% neutral
- 35% disagree

➢ Its possible the LGBT community needs more education on their own health and the issues that might be pertinent to them based on their sexuality and/or gender identity.
### Top 10 Gay Male Health Issues

<table>
<thead>
<tr>
<th></th>
<th>More prominent in LGBT population than other populations</th>
<th>A healthcare issue that is important to me as a patient</th>
<th>My doctor has addressed this issue with me</th>
<th>I wish my doctor would address this issue with me</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Safe sex practices</td>
<td>66%</td>
<td>69%</td>
<td>41%</td>
<td>7%</td>
</tr>
<tr>
<td>1. HIV/AIDS</td>
<td>76%</td>
<td>62%</td>
<td>55%</td>
<td>7%</td>
</tr>
<tr>
<td>2. Drug abuse</td>
<td>41%</td>
<td>31%</td>
<td>21%</td>
<td>3%</td>
</tr>
<tr>
<td>3. Depression/anxiety</td>
<td>38%</td>
<td>69%</td>
<td>41%</td>
<td>10%</td>
</tr>
<tr>
<td>4. Hepatitis immunization</td>
<td>28%</td>
<td>48%</td>
<td>34%</td>
<td>14%</td>
</tr>
<tr>
<td>5. STDs</td>
<td>52%</td>
<td>66%</td>
<td>45%</td>
<td>10%</td>
</tr>
<tr>
<td>6. Prostate/testicular cancer</td>
<td>7%</td>
<td>62%</td>
<td>41%</td>
<td>28%</td>
</tr>
<tr>
<td>6. Colon/anal cancer</td>
<td>17%</td>
<td>55%</td>
<td>31%</td>
<td>21%</td>
</tr>
<tr>
<td>7. Alcohol use</td>
<td>31%</td>
<td>34%</td>
<td>31%</td>
<td>0%</td>
</tr>
<tr>
<td>8. Tobacco use</td>
<td>21%</td>
<td>31%</td>
<td>28%</td>
<td>0%</td>
</tr>
<tr>
<td>9. Fitness</td>
<td>14%</td>
<td>62%</td>
<td>52%</td>
<td>7%</td>
</tr>
<tr>
<td>10. HPV (anal papilloma)</td>
<td>31%</td>
<td>52%</td>
<td>14%</td>
<td>17%</td>
</tr>
</tbody>
</table>
Top 10 Lesbian Health Issues

<table>
<thead>
<tr>
<th></th>
<th>More prominent in LGBT population than other populations</th>
<th>A healthcare issue that is important to me as a patient</th>
<th>My doctor has addressed this issue with me</th>
<th>I wish my doctor would address this issue with me</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Breast cancer</td>
<td>3%</td>
<td>43%</td>
<td>74%</td>
<td>3%</td>
</tr>
<tr>
<td>2. Depression/anxiety</td>
<td>11%</td>
<td>31%</td>
<td>71%</td>
<td>3%</td>
</tr>
<tr>
<td>3. Cardiovascular/heart health</td>
<td>0%</td>
<td>34%</td>
<td>69%</td>
<td>3%</td>
</tr>
<tr>
<td>4. Gynecological cancer</td>
<td>3%</td>
<td>43%</td>
<td>66%</td>
<td>6%</td>
</tr>
<tr>
<td>5. Fitness</td>
<td>6%</td>
<td>40%</td>
<td>83%</td>
<td>3%</td>
</tr>
<tr>
<td>6. Tobacco use</td>
<td>6%</td>
<td>20%</td>
<td>40%</td>
<td>0%</td>
</tr>
<tr>
<td>7. Alcohol use</td>
<td>11%</td>
<td>17%</td>
<td>49%</td>
<td>0%</td>
</tr>
<tr>
<td>8. Drug abuse</td>
<td>11%</td>
<td>14%</td>
<td>26%</td>
<td>0%</td>
</tr>
<tr>
<td>9. Domestic violence</td>
<td>6%</td>
<td>11%</td>
<td>20%</td>
<td>9%</td>
</tr>
<tr>
<td>10. Osteoporosis</td>
<td>3%</td>
<td>26%</td>
<td>66%</td>
<td>9%</td>
</tr>
</tbody>
</table>
Advice to Future Doctors

“Dear Future doctors, for ALL your patients, but especially those belonging to minorities, sexual, racial, ability, religious, or any other... please, make it your practice to ASK and LISTEN before diagnosing, proposing treatment or advising the patient. Your patient is the most knowledgeable person alive about their history, practices, and physical self—valuable information without which you will be handicapped in providing appropriate and effective care.”
Future Possibilities

• Provide participant’s “advice to future doctors” to the Doctoring course directors and/or interested students
• Provide findings to local LGBT and/or healthcare groups
• Service projects to educate the LGBT community on health issues pertinent to them
• 79% said they would like a listing of LGBT-friendly physicians
References


The Effect of Eltrombopag on Human Platelet Resistance to Apoptosis: The Role of the Bcl-xL Pathway

W. Beau Mitchell, MD, Michele N Edison, BS, Mariana P Pinheiro, MD, Nayla Boulad, BA, Bethan Psaila, MD, PhD, Marissa Karpoff, BS, David Kaplan, MD, Benjamin T Kile, PhD, Michael J White, Emma C Josefsson and James B. Bussel, MD
Disclosure

• This study received research support from GlaxoSmithKline. I have a financial relationship with GSK.
Immune Thrombocytopenic Purpura (ITP)

• Immune thrombocytopenia is a disease characterized by:
  – Increased platelet destruction
  – Reduced platelet production

• Mechanism
  – Autoimmune
Immune Thrombocytopenic Purpura (ITP)

- Clinical Presentation
  - Petechiae
  - Purpura
  - Abnormal and exaggerated bleeding tendencies (e.g. after teeth brushing, with menstruation, etc.)

- Affects children and adults differently
Immune Thrombocytopenic Purpura (ITP)

• No cure
• Goal = increase platelet (PLT) count
• Standard Treatments:
  – Corticosteroids
  – Dexamethasone
  – IVIG
  – Splenectomy
  – Platelet transfusions
• Newer Therapies
  – Thrombopoietin receptor (TPO-R) agonists
Eltrombopag (Promacta®)

• TPO-R agonist

• Increases PLT counts in ITP patients

• Stimulates thrombopoiesis

• Effect on platelet lifespan?

• Premature increases in PLT counts
Hypothesis

• We hypothesized that any increase in platelet count in the first week of treatment might be due to effects of Eltrombopag on platelet survival.

• Therefore, this open-label drug trial explored whether Eltrombopag treatment has anti-apoptotic effects in patients with ITP.
Apoptotic Pathway

- Ratio of Bcl-x<sub>L</sub> to Bak determines platelet survival
- Bcl-x<sub>L</sub> expression regulated by TPO-mediated activation of Akt pathways through Jak2 and Stat 5.
Inclusion / Exclusion Criteria

• Inclusion
  – \( \geq 18 \) years
  – ITP by ASH/BCSH guidelines
  – PLT \( \leq 30,000/\mu\text{L} \)
  – Prior response to a prior ITP therapy

• Exclusion
  – Active infection
  – Certain concomitant ITP medications
  – Pregnant, nursing
  – Thrombosis or on blood thinners
  – Active non-dermatologic malignancy
  – Certain laboratory parameters
Methods Overview

• Washout period
• 75 mg Eltrombopag qd
• The following platelet assays were measured at days 1, 3, 5, 8, 10, 12, and 15:
  – CBC With Differentials
  – Immature platelet fraction (IPF)
  – Glycocalicin index
  – Measurement of Bcl-x<sub>L</sub> and other apoptotic pathway proteins from by western blot
  – Measurement of Bcl-x<sub>L</sub> and other apoptotic pathway proteins by intracellular FACS
  – Platelet killing assay
  – Microparticles
Results – Platelet counts

- Platelet counts over time for different patients, showing trends and data points.

- Graph with x-axis representing Day of Treatment and y-axis representing Platelet Count (\( \times 10^9 \) cells/L).

- Patients 1 to 9 are differentiated by distinct markers.

- Mean and Median lines indicated for comparison.

- Image of blood cells in the background.
Results – Platelet counts

![Graph showing large platelet counts over days of treatment for different patients.](image-url)
Results – Platelet counts
Results – Platelet counts

![Graph showing immature platelet fraction over the course of treatment for different patients. The graph includes lines representing individual patients and a red line representing the mean.](image-url)
Results – ABT-737
Results – FACS
Discussion

• Transient increase in Akt may be due to an increase in platelet lifespan
• Platelets more resistant to apoptosis when anti-apoptotic factors are highest regardless of the increase in pro-apoptotic factors.
• Without all of the data, the results are unclear.
Questions?
References

Acknowledgments

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  – Cornell Medical College

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  – The Walter and Eliza Hall Institute for Medical Research, Royal Melbourne Hospital, Australia

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  – Institute of Pathology, CWRU, Ohio

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  – University of Southern California, Department of Medicine

• J.H Beer, M.D.
  – Kantonsspital, Department of Medicine, Laboratory for Thrombosis Research, Baden, Switzerland

• Keith R. McCrae, M.D.
  – Cleveland Clinic, Lerner Research Institute, Ohio
Background Information

• Csm1/Lsr4 is a DNA clamp protein which serves several functions in the budding yeast cell, including:
  – homolog segregation in meiosis I
  – localization of rDNA and telomeres to the nuclear envelope
  – Essential in mono-orientation of kinetochores as part of the monopolin complex.
  – Regulating DNA silencing at rDNA via interactions with RENT complex.

Our goal is to investigate the role of Csm1 and Lrs4 in chromosome segregation in mitosis.
Background Information
Cohesin & Cohesion

[Diagram showing the process of DNA replication and cell division, with stages labeled as S, G2, and M.]
Research Objectives

Our goal was to investigate the role of Csm1/Lrs4 proteins in mitosis.

• Compare cell cycle progression in wild-type and csm1Δ, lrs4Δ mutant cells.
• Confirm the sister centromere reassociation defect in nocodazole-treated csm1Δ and lrs4Δ mutants.
• To examine the re-establishment of chromosome bi-polar attachment in csm1Δ and lrs4Δ mutant cells after nocodazole treatment.
• Perform a Chromatin immunoprecipitation to analyze cohesin binding to centromere in csm1Δ and lrs4Δ mutant strains.
Comparing Cell Cycle Progression

Percentage of large budded cells over time in alpha-factor treated cells

- Graph showing the percentage of large budded cells over time for different treatments.
- The x-axis represents time in hours, while the y-axis represents the percentage of large budded cells.
- Different lines represent different treatments, with a legend indicating their characteristics.
Confirming the Reassociation Defect
Confirming the Reassociation Defect

Two-tailed p-values

<table>
<thead>
<tr>
<th></th>
<th>90 MINUTES</th>
<th>150 MINUTES</th>
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</thead>
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<td>wt:csM1</td>
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<td>&lt;0.0001</td>
</tr>
<tr>
<td>wt:lrs4</td>
<td>&lt;0.0001</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>
Synchronous wild type cells 2 hours after nocodazole treatment
Synchronous csm1Δ cells 2 hours after nocodazole treatment
Bi-Polar Re-orientation Assessment
Bi-Polar Reorientation Assessment

wild type cells

The microtubules (labelled in red) appear to be aligned with the sister chromatids and appear to be in various states of segregation, moving to opposite spindle pole bodies.
Bi-Polar Orientation Assessment

mutant cells

These are examples of erroneous microtubule disassociation. In the two images on the left, the spindle is fully elongated but both GFP signals are localized to one of the buds. The two images on the right have spindles that are not running in an axis that would allow for chromosome segregation.
**ChIP Analysis**

<table>
<thead>
<tr>
<th></th>
<th>1: non-tagged</th>
<th>2: Lrs4-myc</th>
<th>3: Csm1-myc</th>
<th>4: Nnf1-myc</th>
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</thead>
<tbody>
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<td><img src="image-url" alt="Chip Band" /></td>
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<td><img src="image-url" alt="Band 2" /></td>
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<td><img src="image-url" alt="Band 3" /></td>
<td><img src="image-url" alt="Band 4" /></td>
</tr>
</tbody>
</table>
ChIP Analysis

ChIP Quantification of protein binding to CEN3

binding capacity (scaled)

protein

no tag (negative control)  Lrs4  Csm1  Nnf1 (positive control)

CEN3
Conclusions

• There is a cell cycle delay in ΔCsm1 and ΔLrs4 mutants in comparison to the wild type strain.

• Csm1 and Lrs4 are involved in sister chromatid interactions.

• Although not as evident as proteins that associate directly with centromeric DNA, the csm1 and lrs4 protein do has some interaction with centromeric DNA based on ChIP analysis.
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• The countless number of yeast that I killed over the summer
References


