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Award Number: W81XWH-11-1-0731

TITLE: Modulation of Estrogen-Depurinating DNA Adducts by Sulforaphane for Breast Cancer

PRINCIPAL INVESTIGATOR: Dr. Li Yang

CONTRACTING ORGANIZATION: University of Pittsburgh
PITTSBURGH, PA 15213-3320

REPORT DATE: October 2012

TYPE OF REPORT: Annual Summary

PREPARED FOR: U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE	2. REPORT TYPE	3. DATES COVERED
01-10-2012	Annual Summary	15 Sep 2011 - 14 Sep 2012

4. TITLE AND SUBTITLE
Modulation of Estrogen-Depurinating DNA Adducts by Sulforaphane for Breast Ccancer

5a. CONTRACT NUMBER

5b. GRANT NUMBER
W81XWH-11-1-0731

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
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5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
UNIVERSITY OF PITTSBURGH

PITTSBURGH PA 15213-3320

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
U.S. Army Medical Research and Materiel Command
Fort Detrick, Maryland 21702-5012

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT
Approved for Public Release; Distribution Unlimited

13. SUPPLEMENTARY NOTES

14. ABSTRACT Sulforaphane (SFN), a bioavailable phytochemical found in young broccoli, is a potent inducer of detoxification enzymes such as NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-transferase (GST) via the Kelch-like ECH-associated protein 1 (Keap1) - Nuclear Factor- E2-related factor (Nrf2) signaling pathway. To test the hypothesis that SFN may be an ideal chemoprevention agent to block estrogen-mediated carcinogenesis, we treated the ER-negative, nontumorigenic human breast epithelial MCF10A cell line with either vehicle or SFN (10μM) and E_2 or 4-OHE_2. Results show that NQO1 was up-regulated at the mRNA (~2fold), protein (~3fold) and activity levels (~3fold) by SFN treatment. Estrogen metabolites and depurinating DNA adducts in the cell culture medium were partially purified by solid phase extraction and then analyzed by UHPLC- ESI-MS/MS. Following E_2 treatment, the depurinated adducts 4-$OHE_{1/2}$-1-N3Ade and 4-$OHE_{1/2}$-1-N7Gua were significantly lower in SFN treated cells compared to vehicle (0.03±0.01 versus 0.07±0.02 pmole/10^6cell, p=0.0294); 4-$OHE_{1/2}$- glutathione conjugates were significantly higher following SFN treatment (1.54±0.37 versus 0.83±0.19 pmole/10^6cell, p=0.0015) as were 4-$OCH_3E_{1/2}$ (5.36 ± 0.16 versus 1.81±0.20pmole/10^6cell,p<0.0001) levels. Following treatment with the proximate metabolite 4-OHE_2, 4-$OHE_{1/2}$-1-N3Ade and 4-$OHE_{1/2}$-1-N7Gua were again significantly lower in SFN treated cells compared to vehicle (0.59±0.11 versus 1.42±0.16 pmole/10^6cell, p=0.0028) while 4-$OHE_{1/2}$-glutathione-conjugates (4.44±0.52 versus 0.87±0.03 pmole/10^6cell,p=0.0001) and 4-$OCH_3E_{1/2}$ levels were significantly higher (195.00±12.33 versus 58.05±1.77pmole/10^6cell, p<.00001).

15. SUBJECT TERMS
Sulforaphane; NQO1;GST;estrogen depurinating DNA adducts;Keap1-Nrf2; breast cancer; chemoprevention; UHPLC-MS/MS

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			USAMRMC
U	U	U	UU	43	19b. TELEPHONE NUMBER *(include area code)*

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Introduction

My long-term career goal is to be a leader in basic and translational breast cancer research, and have an impact on the prevention of breast cancer. To achieve the goal of this journey, a valuable postdoctoral training in breast cancer research in a setting with experienced, well established investigators is very important. The BC103928 Postdoc Fellowship Award granted me the opportunity to deepen my understanding of the mechanisms and underlying role of estrogens in the process of carcinogenesis leading to breast cancer. The goal of this study is to investigate the modulation of estrogen-depurinating DNA adducts by sulforaphane for breast cancer chemoprevention. I am under the supervision of a Mandatory Career Development Committee composed of Dr. Thomas Kensler (mentor), Dr. Nancy Davidson (co-mentor), Dr. Bruce Freeman and Dr. Kala Visvanathan. With this training, I will gain further experience in cell culture, animal models, clinical samples, mass spectrometry and estrogen metabolism at University of Pittsburgh.
In addition to learning laboratory techniques, I will attend local, national and international meetings and workshops, classes and seminars that relate to mass spectrometry and clinical trial methods and practice. I have the opportunity to receive guidance from both my meetings with the Career Development Committee and from communications with other senior experts in breast cancer research in the Department of Pharmacology such as Dr. Steffi Oesterreich and Dr. Adrian Lee. In summary, this is a comprehensive and personalized training program to ensure the highest productivity and to effectively facilitate my career goal of transitioning into an independent breast cancer researcher.

Body

In the past year, I have been working on research and training tasks according to the original schedule.
I have established a solid phase extraction method to partially purify estrogen metabolites and depurinating DNA adducts from cell culture medium. I have developed a UHPLC-MS/MS method to separate and quantify estrogen metabolites and depurinating DNA adducts and used this method to quantify levels in MCF-10A cell culture media treated with vehicle or sulforaphane and E_2 or 4-OHE_2.
Sulforaphane (SFN) is a potent inducer of detoxification enzymes such as NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-transferase (GST) via the Kelch-like ECH-associated protein 1 (Keap1) - Nuclear Factor- E2-related factor (Nrf2) signaling pathway. NQO1 reduces the carcinogenic estrogen metabolite, Catechol Estrogen-3,4-Quinone (CE-3,4-Q), to catechols while GSTs detoxify it through nucleophilic addition. CE-3,4-Q can bind with DNA to form depurinating DNA adducts, leading to DNA damage via an estrogen receptor (ER) independent pathway. Thus, SFN may be an ideal chemoprevention agent to block estrogen-mediated carcinogenesis.
To test the above hypothesis, I have treated MCF10A cells with either vehicle or SFN (10 μM) and E_2 (10μM) or 4-OHE_2(10μM). The results show that NQO1 was up-regulated at the mRNA (~2fold), protein (~3fold) and activity levels (~3fold) by SFN treatment. Estrogen metabolites and depurinating DNA adducts in the cell culture medium were partially purified by solid phase extraction and then analyzed by UHPLC- ESI-MS/MS. Following E_2 treatment, the depurinated adducts 4-$OHE_{1/2}$-1-N3Ade and 4-$OHE_{1/2}$-1-N7Gua were significantly lower in SFN treated cells compared to vehicle (0.03±0.01 versus 0.07±0.02 pmole/10^6cell, p=0.0294); 4-$OHE_{1/2}$- glutathione conjugates were significantly higher following SFN treatment (1.54±0.37 versus 0.83±0.19 pmole/10^6cell, p=0.0015) as were 4-$OCH_3E_{1/2}$ (5.36 ± 0.16 versus 1.81±0.20pmole/10^6cell,p<0.0001) levels. Following treatment with the proximate metabolite 4-OHE_2 , 4-$OHE_{1/2}$-1-N3Ade and 4-$OHE_{1/2}$-1-N7Gua were again significantly lower in SFN treated cells compared to vehicle (0.59±0.11 versus 1.42±0.16 pmole/10^6cell, p=0.0028) while 4-$OHE_{1/2}$-glutathione-conjugates (4.44±0.52 versus 0.87±0.03 pmole/10^6cell,p=0.0001) and 4-$OCH_3E_{1/2}$ levels were significantly higher (195.00±12.33 versus 58.05±1.77pmole/10^6cell, p<.00001). In summary of the past one year work, SFN can modulate estrogen metabolism leading to diminished formation of estrogen-DNA adducts.

For the training tasks, I have completed task 1 and 2. I attended "TSQ Family Operations MASS Spectrometry training" at Thermo Scientific in 10/24/2011 – 10/27/2011 in Florida. I have also learned course "Clinical Trials: Methods and Practice" at Graduate School of Public Health in spring 2012 (auditing).

In addition to completing the research and training tasks according to the schedule, I have attended the 24th University of Pittsburgh Cancer Institute 2012 Scientific Retreat in June at Greensburg, PA. I also attended the University of Pittsburgh Cancer Institute 2012 Satellite Conference in June at Greensburg, PA.

Key Research Accomplishments

In the MCF-10A cell study, I found that SFN can modulate estrogen metabolism leading to diminished formation of estrogen-DNA adducts by up-regulating NQO1 and GST via Keap1-Nrf2 pathway. The manuscript is under preparation.

Reportable Outcomes

- 2012, the 1st place poster award for clinical/translational cancer research, the 24th University of Pittsburgh Cancer Institute 2012 Scientific Retreat, Greensburg, PA. Abstract: Modulation of estrogen metabolism and estrogen depurinating DNA adducts via activation of Nrf2 signaling by sulforaphane in human breast epithelial cells. (Poster). Yang, L; Zahid, M., Cavalieri, E.; Rogan, E., Kensler T.
- 2012, the 2nd place poster ward in cancer epidemiology, prevention and control program, University of Pittsburgh Cancer Institute 2012 Satellite Conference, Greensburg, PA. Abstract: Modulation of estrogen metabolism and estrogen depurinating DNA adducts via activation of Nrf2 signaling by sulforaphane in human breast epithelial cells. (Poster). Yang, L; Zahid, M., Cavalieri, E.; Rogan, E., Kensler T.
- Poster presentation at Women's cancer research center Retreat in Farmington, PA in Sep. 2012 (poster title: Modulation of estrogen metabolism and estrogen depurinating DNA adducts via activation of Nrf2 signaling by sulforaphane in human breast epithelial cells.)
- Invited oral presentation at UPCI seminar series in Pittsburgh PA in Sep, 2012 (Title: Pharmacological and genetic activation of Nrf2 signaling in MCF-10A cell for modulation of estrogen depurinating DNA adducts).
- One manuscript is under preparation (Title: Pharmacological and genetic activation of Nrf2 signaling in MCF-10A cell for modulation of estrogen depurinating DNA adducts.)
- Received the training in TSQ MASS SPECTROMETRY at Thermo
- Learned course - Clinical Trials: Methods and Practice
- Review papers for the following journals: Biomarker Insights; Carcinogenesis;Breast cancer: Basic and Clinical Research; Nutrition and Metabolic Insights; Clinical Medicine Insights: Women's Health
- Judge for Intel International Science & Engineering Fair 2012. Pittsburgh, PA, USA

Conclusion

SFN can modulate estrogen metabolism leading to diminished formation of estrogen-DNA adducts via Keap1-Nrf2 pathway in breast epithelial cells. The findings support that SFN, a food-derived natural product, could be a novel breast cancer chemoprevention agent.

References

N/A

Appendices

See the attached.

Curriculum vitae

Li Yang, PhD.

July 2012

Li Yang, PhD.
Department of Pharmacology & Chemical Biology,
University of Pittsburgh School of Medicine,
Pittsburgh, PA, 15261
TEL: (412) 760-6249 (cell)
E-mail: liyang@pitt.edu

Education

PhD in Environmental Toxicology, 2010

Department of Environmental, Agricultural and Occupational Health

College of Public Health, UNMC, Omaha, NE, USA

PhD Dissertation

Estrogen metabolism and risk of breast cancer and prostate cancer: Detection of potential early biomarkers from case-control studies

M.S. in Biology, 2002

Department of Biology Science

College of Biology, China Agricultural University, Beijing, China

B.S. in Plant Protection, 1995 (plant pathology, pesticides)

Department of Horticulture

Inner Mongolia Agricultural University, Inner Mongolia, China

Working Experience

Postdoc. Associate, June 2010 - present:

Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine

PhD student, Aug 2004 – May 2010:

Department of Environmental, Agricultural and Occupational Health

College of Public Health, UNMC, Omaha, NE, USA

Research Associate, July. 2002 - Aug. 2004:

Department of Pesticide, School of Science, China Agricultural University, Beijing, China

Honors/Awards

2012, Award for the 1st place best poster presentation for clinical study, 24th University of Pittsburgh Cancer Institute Scientific Retreat 2012, Greensburg, PA.

2012, Award for the 2nd place best poster presentation, University of Pittsburgh Cancer Institute Satellite Conference 2012, Greensburg, PA.

2011 -2014, DOD breast cancer Postdoc Fellowship Award (Period: 15, SEP, 2011- 14, OCT, 2014. Total funding: $448,770.00. Title of the Grant: Modulation of Estrogen-Depurinating DNA Adducts by Sulforaphane for Breast Cancer chemoprevention.)

2007-2010, UNMC graduate school, Fellowship Award

2009, Award for the 2nd place best abstract, American Association of Chinese in Toxicology (AACT), Baltimore, Maryland.

2009 Award for Graduate Student Travel Support for 2009 National Environmental Public Health Conference, Atlanta, GA.

2008, Award for the 3rd place poster presentation, 39th Midwest Student Biomedical Research Forum, Omaha, Nebraska.

2008, Award for Graduate Student Travel Support for 2008 National Toxicology Conference. Society of Toxicology (SOT). Seattle, Washington.

2008, Representative of UNMC graduate students for International Student Research Forum. Omaha, Nebraska.

2007, Award for best poster presentation, Central States Society of Toxicology Meeting. Iowa City, Iowa.

2004, Special award for contribution to the pesticides research, Society of Pesticide Res., Beijing, China.

Research Presentations

2nd Annual Women Cancer Research Center (WCRC) Retreat 2012 – Sep. 7-8, 2012. Farmington, PA. Modulation of estrogen metabolism and estrogen depurinating DNA adducts via Pharmacological and genetic activation of Nrf2 signaling in human

breast epithelial cells. (Poster). **Yang, L**; Zahid, M., Cavalieri, E.; Rogan, E., Kensler T.

Metabolomics Society 8th Annual Meeting 2012 – June 25-28, 2012. Washington, DC. Determination of serum estrogen depurinating DNA-adducts as potential biomarker for breast cancer risk: results from a case-control study. (Abstract 402). **Yang, L**; Kensler, T; Cavalieri, E.; Rogan, E. et al.

24th University of Pittsburgh Cancer Institute Scientific Retreat 2012 – June 21 – 22, 2012. Greensburg. Modulation of estrogen metabolism and estrogen depurinating DNA adducts via activation of Nrf2 signaling by sulforaphane in human breast epithelial cells. (Poster). **Yang, L**; Zahid, M., Cavalieri, E.; Rogan, E., Kensler T.

University of Pittsburgh Cancer Institute Satellite Conference 2012 (Cancer Epidemiology, Prevention and Control Program) – June 20, 2012. Greensburg. Modulation of estrogen metabolism and estrogen depurinating DNA adducts via activation of Nrf2 signaling by sulforaphane in human breast epithelial cells. (Poster). **Yang, L**; Zahid, M., Cavalieri, E.; Rogan, E., Kensler T.

First annual women's cancer and research center retreat at UPMC – May 6-7, 2011, Pittsburgh, PA. (Poster 16). The role of estrogen metabolism in risk of developing breast cancer: Detection of novel serum biomarkers from a case-control study. **Yang L**, Kensler T., Rogan E. G. et al.

Society of Toxicology 50th Annual meeting 2011 – March 6-10, 2011, Washington D.C. Plateform presentation. (Abstract 880). Associtation of estrogen metabolism and risk of non-hodgkin lymphoma: detection of novel biomarkers from case-control study. **Yang L**, Gaikwad W. N., Cavalieri E. L. and Rogan E. G. et al.

Society of Toxicology 49th Annual meeting 2010 – March 7-11, 2010, Salt Lake City, Utah. Poster presentation. (1885 Poster Board -440). Component analysis of novel serum biomarker of breast cancer: results from case-control studies. **Yang L**, Cavalieri E. L. and Rogan E. G. et al.

The 41st Midwest Student Biomedical Research Forum – February 20, 2010, Omaha, NE. Oral Presentation. Association between novel serum biomarkers for assessing breast cancer risk and Gail model score: Results from a case-control study. **Yang L**., Rogan E.G., Cavalieri E. L., and Pruthi S.

Society of Toxicology 48th Annual meeting 2009 – March 15-19, 2009, Baltimore, Maryland. Poster presentation (abstract 1605 at poster board 514): Novel urinary

biomarkers for risk of prostate cancer: results from a case-control study. **Yang L**, Cavalieri E. L. and Rogan E. G.

The 40th Midwest Student Biomedical Research Forum – February 28 - 29, 2009, Omaha, NE. Oral Presentation: Novel serum biomarkers for assessing breast cancer risk: results from a case-control study in USA. **Yang L**., Rogan E.G., Cavalieri E. L., and Pruthi S.

International Student Research Forum. June 1-3, 2008, Omaha, NE. Oral presentation: Estrogen metabolism and risk of breast cancer: detection of novel biomarkers from case-control study. **Yang L**, Rogan E. G and Cavalieri E. L.

Society of Toxicology 47th Annual meeting 2008– March 16-20, 2008, Seattle, Washington. Poster presentation #1735&VSP#3009: The role of estrogen metabolism in the initiation of breast cancer: Detection of potential early biomarkers from case-control studies. **Yang L**, Cavalieri E. L. and Rogan E. G. et al.

The 39th Midwest Student Biomedical Research Forum – February 29- March1, 2008. Omaha. Poster Presentation P-50: The role of estrogen metabolism in the risk of developing breast cancer: Detection of novel serum biomarkers from a case-control study". **Yang L**, Rogan E. G and Cavalieri E. L.

30th Annual San Antonio Breast Cancer Symposium. Dec. 13-16, 2007. Texas. Poster #5001. Molecular etiology of breast cancer: potential biomarkers of risk and for use in prevention. Cavalieri E. L., Gaikwad N, **Yang L**, et al.

Central States Society of Toxicology Meeting – September 20-21, 2007, University of Iowa Medical Center. Poster Presentation: The role of estrogen metabolism in the initiation of breast cancer: Detection of potential early serum biomarkers from a case-control study. **Yang L**, Rogan E. G and Cavalieri E. L.

The 38th Midwest Student Biomedical Research Forum – February 23, 2007. Omaha. Poster Presentation P-47: "Mass spectrometry detection of a novel biomarker of breast cancer in human serum". **Yang L**, Rogan E. G and Cavalieri E. L.

The 37th Midwest Student Biomedical Research Forum – February 18, 2006. Omaha. Poster Presentation P-68: "Mass spectrometric analysis of potential biomarkers of breast cancer in human serum". **Yang L**, Gaikwad N, Rogan E. G and Cavalieri E. L.

Note: Even though the title of the above posters might be the same or similar, the contents of above posters are different because the sample size increased with time and all the data were presented with updated data.

Membership

- Active Member, American Association of Cancer Research (AACR), 2011-present
- Member, American Society for Mass Spectrometry, 2011-present
- Member, Women in Toxicology (WIT) SIG, 2010 – present
- Member, American Association of Chinese in Toxicology (AACT) Special Interest Group (SIG), 2009 - present
- Full Member, Society of Toxicology (SOT), 2011 – present
- Graduate student SOT member, 2008 - 2011

Publication

Yang L. Kensler T, Cavalieri E., Rogan E., Zahid M. Pharmacological and genetic activation of Nrf2 signaling in MCF-10A cell for modulation of estrogen depurinating DNA adducts. (in preparation).

Yang L, Cavalieri EL, Rogan EG. Ingle J., Pruthi S., Association between novel serum biomarkers for assessing breast cancer risk and Gail model score: Results from a case-control study (in preparation).

1. Pruthi S.*, **Yang L ***, Ingle J., Sandhu N., Suman V., Cavalieri E. L., Rogan E. G. (**2012**). Evaluation of serum estrogen-DNA adducts as potential biomarkers for breast cancer risk. J Steroid Biochem Mol Biol. *(* equal contribution as first author*). 2012 Feb 24. [Epub ahead of print]. PMID: 22386952.

2. Zahid M, Saeed M, **Yang L**, Beseler C, Rogan EG, Cavalieri EL. (**2011**). Formation of dopamine quinone-DNA adducts and their potential role in the etiology of Parkinson's disease. International Union of Biochemistry and Molecular Biology. 63(12):1087-93.

3. Gaikwad NW, **Yang L**, Weisenburger DD, Vose J, Beseler C., Rogan E.G. and Cavalieri E. L. (2009) Urinary biomarkers suggest that estrogen-DNA adducts may play a role in the aetiology of non-Hodgkin's lymphoma Biomarkers. 14(7): 502-12.

4. Maddalena Barba, **Yang L**, Francesca Sperati, Holger J. Schünemann, Sara Grioni, Saverio Stranges, Kim C. Westerlind, Michele Gallucci, Paola Muti. (**2009**). Urinary estrogen metabolites and prostate cancer: a case–control study and meta-analysis. Journal of Experimental & Clinical Cancer Research. 28:135.

5. **Yang L**, Gaikwad NW, Cavalieri EL, Muti P, Trock B, Rogan EG. (**2009**) Novel biomarkers for risk of prostate cancer: Results from a case-control study. The Prostate. 69:41-48.

6. Gaikwad NW, **Yang L**, Pruthi S., Ingle J., Rogan E.G. and Cavalieri E. L.(**2009**) Urinary biomarker of risk in the molecular etiology of breast cancer. Breast cancer: basic and clinical research. 3:1-8.

7. Gaikwad NW, **Yang L**, Rogan E.G. and Cavalieri E. L. (**2009**). Evidence for NQO2-mediated reduction of the carcinogenic estrogen ortho-quinones. Free Radic Biol Med. 46(2):253-62.

8. Gaikwad NW, **Yang L**, Muti P., Meza J., Ingle J., Pruthi S., Rogan E.G. and Cavalieri E. L.. (**2008**). The molecular etiology of breast cancer: evidence from biomarkers of risk. Int J Cancer. 122(9):1949-57.

9. Zahid M, Gaikwad NW, Ali MF, Lu F, Saeed M, **Yang L**, Rogan EG, Cavalieri EL. (**2008**). Prevention of estrogen-DNA adduct formation in MCF-10F cells by resveratrol. Free Radic Biol Med. 45(2):136-45.

10. **Yang L**., Liao Y., Wang P., Bi C.L., Zhou Z.Q., Jiang S.R. (**2004**) Direct optical resolution of chiral pesticides by high performance liquid chromatography on cellulose tris-3, 5-dimethylphenyl carbamate stationary phase under reversed phase conditions. Journal of Liquid Chromatography & Related Technologies. 27(18): 2935-2944.

11. **Yang L**., Liao Y., Zhou Z.Q., Jiang S.Q., Wang P. (**2004**) Separation of enantiomers of two pesticides using β-CD as a chiral mobile phase additive in high-performance liquid chromatography. Chinese Journal of Pesticide Science. 6(2): 90-92.

12. **Yang L**., Jiang S.R., Liao Y., Wang P., Tian Q., Zhou Z.Q. (**2004**) Chiral separation of hexaconazole by reversed phase HPLC with β-cyclodextrin as a mobile phase and normal phase HPLC with CDMPC as chiral stationary phase. Journal of Instrumental Analysis. 23(5):133 -135.

13. Wang P., Zhou Z.Q., Jiang S.R., **Yang L**. (**2004**) Chiral resolution of cypermethrin on cellulose-tris(3,5-dimethylphenyl-carbamate) chiral stationary phase. Chromatographia. 59 (9-10): 625-629.

14. Zhao H.X., Qiu Y.M., Wang L.P., Zhou Z.Q., **Yang L**. (**2004**) Research development of the determination of barbiturates. Animal Science & Veterinary Medicine (Chinese) Vol.21 No.03 P.29-30.

15. Zhou Z.Q., Wang P., Jiang S.R., Wang M., **Yang L**.(**2003**). Preparation of polysaccharide-based chiral stationary phases and the direct separation of six chiral pesticides and related intermediates. Journal of Liquid Chromatography & Related Technologies. 26(17): 2873-2880.

16. Wang P., Zhou Z.Q., Jiang S.Q., **Yang L**., Zhang H.J. (**2003**) The preparation of two coated cellulose-based chiral stationary phases and the direct resolution of chiral pesticides and related intermediates. 2nd International Academic Symposium on Pesticides and Environmental Safety (Beijing) 175-180.

Research and Laboratory Skills

- Chemical analysis: UPLC/MS-MS; HPLC; MALDI-TOF; Chiral pesticides separation
- Statistical software: PAWS; SPSS; SAS; Prizm
- Biology: Cell culture; western blotting; PCR; Animal model
- Epidemiology: design and execution of studies (cohort, case control, and cross-sectional studies); environmental epidemiology of cancer, epidemiological data analysis
- Purification of biological fluid samples (including serum, urine and cell culture medium)
- Solid phase extraction technique

Teaching Experience

- Teaching Assistant in the course: Plant cell anatomy (Sep. 2000 – May, 2001. China Agricultural University, 33 undergraduate student, major in plant protection)
- English teacher (Aug. 2000. – May, 2001, Peking PeiLi ZhiYe College, 30 undergraduate student, major in English)

Information Technology Skills

- Molecular Modeling software
- Microsoft Word, Excel, and PowerPoint

- Basic Internet skills

Invited Presentations

2008. Novel serum biomarkers for assessing breast cancer risk: results from a case-control study. For Breast Cancer Training Program at Eppley Cancer Center, UNMC, Omaha, NE.

2008. The role of estrogen metabolism in the initiation of breast cancer: Detection of potential early biomarkers from case-control studies. Society of Toxicology 47th Annual meeting. Special session for visiting students, Seattle, Washington.

2012. Pharmacological and genetic activation of Nrf2 signaling in MCF-10A cell for modulation of estrogen depurinating DNA adducts. University of Pittsburgh Cancer Institute. Pittsburgh, PA.

Invited Reviews

2011- 2012. Biomarker Insights. (Four times)
2011. Carcinogenesis (One time)
2011- 2012. Breast cancer: Basic and Clinical Research (Three times)
2011. Nutrition and Metabolic Insights (One time).
2011. Clinical Medicine Insights: Women's Health (One time).

Invited Judgment

2012. Intel International Science & Engineering Fair 2012. Pittsburgh, PA, USA.

Training record:

From: us.training.analyze@thermofisher.com <us.training.analyze@thermofisher.com>
Subject: Thermo Scientific Enrollment Confirmation
Date: Fri, October 7, 2011 1:15 pm
To: liyang@pitt.edu <liyang@pitt.edu>

Dear LI YANG:

This is your course confirmation! We would like to thank you for enrolling in the following course(s):
TSQ Family Operations
10/24/2011: 9:00 AM - 5:00 PM
10/25/2011: 9:00 AM - 5:00 PM
10/26/2011: 9:00 AM - 5:00 PM
10/27/2011: 9:00 AM - 5:00 PM

Location: West Palm Beach
1400 North Point Parkway Suite 10 West Palm Beach, Florida 33407
Room: Key Largo

Enrollment Status: Enrolled

Thank you! If you are paying by credit card, please call 1-800-532-4752.

Please note by the submission of your on-line Pre-Registration you agree to adhere to our cancellation, Weather Related and refund policy that is list below: Cancellation Policy ·We reserve the right to cancel any course, 30 calendar days prior to the scheduled start date, due to insufficient enrollment.·We reserve the right to change the venue of the course, 30 calendar days prior to the scheduled start date.Travel arrangements should not be made more than 30 calendar days in advance, as the venue is subject to change. ·In the event of a venue change, you will be notified by a Thermo representative. Thermo will not be responsible for expenses incurred (for example, non-refundable airline reservations) if the course is cancelled or moved.·Cost for courses is quoted in U.S. dollars.·Payment in full is due upon course commencement.·Attendee substitutions may be made at any time (call us first to determine if course is appropriate).·Enrollment in your desired training course(s) is not guaranteed until receipt of this form, the pre-course questionnaire (if applicable), and confirmed method of payment. Weather Related Cancellation ·We reserve the right to cancel any course, by 2pm Eastern time the Friday prior to the course start date.·Thermo will not be responsible for expense incurred (for example, no-refundable airline reservations).·If a customer arrives to the area in advance and the course is cancelled by 2pm Eastern the Friday prior to the course start date; Thermo is not responsible for expenses incurred.·For weather advisories, please contact us at 800-532-4752 and speak directly with the registrar. Refund Policy ·100% refund for cancellations received 15+ business days prior to course date.·50% refund for cancellations received 10-15 business days prior to course date.·No refund for cancellations received fewer than 10 business days prior to course date.·No refund for no-shows.
If you have any questions or need further information please feel free to contact us.

Class content

BIOSTATISTICS 2062

Clinical Trials: Methods and Practice

Lecture Schedule
Spring Term, 2012 (12-2)
Class Times: Thursday, 9:00 am - 11:50 am
Room: A425 Crabtree Hall, GSPH

Date	Topics	Instructor
1/05/12	Introduction and History of Clinical Trials	Dr. Redmond
	Salk Vaccine Clinical Trial	Dr. Redmond
1/12/12	Ethical Considerations	Dr. Redmond
	Clinical Trials Organization and Protocol Development	Dr. Redmond
1/19/12	Translational Studies	Dr. Redmond
	Phase I - Dose Finding Designs and Safety Assessment	Dr. Redmond
2/02 /12	Randomization and Treatment Allocation Methods	Dr. Redmond
	First Examination	
2/09/12	Bias, Precision, and Generalizability	Dr. Rockette
	Overview of Clinical Trial Designs	Dr. Rockette
2/16/12	Comparative Trials: Parallel Group Designs	Dr. Redmond
	Comparative Trials: Factorial and Crossover Design	Dr. Redmond
2/23/12	Comparative Trials: Cluster Randomized Designs	Dr. Redmond
	Comparative Trials: Equivalence Trials	Dr. Redmond
2/26/12	Phase II - Activity/Efficacy Studies	Dr. Redmond
	Sample Size Considerations	Dr. Redmond
3/01/12	Multiple Comparisons and Multiplicity	Dr. Rockette
	Collaborative Group Projects: Introduction	Dr. Redmond

Spring Break - March 5-9, 2012

Date	Topics	Instructor
3/15/12	Time to Event Outcomes – Life Table Methods	Dr. Rockette
	Time to Event Outcomes – Regression Models	Dr. Rockette
3/22 /12	General Analysis Considerations; Continuous and Discrete Outcomes	Dr. Redmond
	Second Examination	Dr. Redmond
3/29/12	Repeated Measures in Clinical Trials	Dr. Redmond
	Controlling for Confounding; Testing for Interaction	Dr. Redmond
4/05/12	Intention-to-Treat Analysis: Rationale and Approaches	Dr. Redmond
	Interim Data Analysis	Dr. Redmond
4/12/12	Final Analysis and Reporting Guidelines	Dr. Redmond
	Meta-analytic Methods	Dr. Redmond
4/19/12	Collaborative Group Presentations	Students

rom: "Redmond, Carol K" <ckr3@pitt.edu>
Subject: RE: hello from li yang
Date: Fri, April 27, 2012 3:49 pm
To: "Yang, Li" <liyang@pitt.edu>

Dear Li Yang,

It was a pleasure to have you in the class. Your contributions as an auditor were important. I hope that you will keep in touch and let me know about how your career and other activities progress over time. If you should need a statistical collaborator or consultant at some time in the future, please feel free to contact me. If I am not able to assist directly, I will try to recommend someone to you.

Best wishes and warm regards,

Carol Redmond

Dr. Carol K. Redmond

Distinguished Service Professor of Public Health

318A Parran Hall, University of Pittsburgh

130 DeSoto Street

Pittsburgh, PA 15261
Telephone: 1-412-624-0765 Fax: 1-412-624-2183
http://www.biostat.pitt.edu/redmond.htm
http://www.wiley.com/legacy/products/subject/reference/bct_articles.html

Abstract acceptance record

University of Pittsburgh Cancer Institute

WOMEN'S CANCER RESEARCH CENTER

- Welcome
- Agenda
- Register Now
- Abstract Info

Welcome

Dear WOMEN'S CANCER RESEARCH CENTER MEMBERS:

We are pleased to invite you to attend the **2nd Annual WCRC Retreat** on September 7 and 8, 2012 at Nemacolin Woodlands Resort (1001 Lafayette Drive, Fa of the Retreat are to showcase exciting research which is currently performed in labs of WCRC members, to further enhance interaction, and foster collaboratio stimulating program in the area of women's cancer research, covering basic, clinical, and translational areas of investigations.

The Retreat will begin Friday September 7th at noon, with a presentation by Dr Lee, Director of the WCRC, entitled *"Two years of WCRC - what have we acco done?"*. For the afternoon, we have scheduled presentations on breast and ovarian cancer topics (including presentations by last year's WCRC Pilot grant award conclude with dinner and entertainment. The Keynote Address will be given by Dr Bill Hahn, Associate Professor, Department of Medicine, Harvard Medical S Cancer Genome Discovery, Dana-Farber Cancer Institute. Dr Hahn is an expert in cancer genomics (including breast and ovarian cancer), and high-throughput Saturday session will include a short morning session for all and then interested Faculty can participate in a brain-storming session dealing with programmatic i

We very much look forward to your attendance and participation in this retreat. We especially encourage you to bring students and fellows, as our trainees are s current and future research endeavors! We will need to limit the number of attendees at the Retreat, and therefore encourage you to register soon. Should we rea Retreat committee will make decisions based on active participation in WCRC activities.

All information, including a preliminary program, and forms regarding the WCRC Retreat can be found at http://www.upci.upmc.edu/WCRC/retreat. Please use abstract submission forms for this year's event. **The deadline for registration (including abstract submission) will be July 10th at 5 pm**. For questions regar Steffi Oesterreich, oesterreichs@upmc.edu.

Adrian V Lee, PhD
Director, WCRC

Bob Edwards, MD
Co-Director, WCRC

IN PARTNERSHIP WITH:

From: kpater@magee.edu

Subject: Notice of Acceptance: 2012 WCRC Retreat Abstract Submission

Date: Tue, July 10, 2012 9:54 am

To: liyang@pitt.edu

Congratulations! Your abstract has been accepted for display and presentation at the Women's Cancer Research Center Retreat on September 7, 2012. You will be notified of your poster number the week before the retreat.

If you have any questions regarding your abstract submission or the UPCI Scientific Retreat, please contact Kathleen Pater at kpater@magee.edu
.

Invited oral presentation record

From: "Bakkenist, Christopher J" <bakkenistcj@upmc.edu>
Subject: UPCI seminar for UPCI retreat poster winners (Clinical Science)
Date: Fri, July 6, 2012 2:04 pm
To: "nakajima.erica@medschool.pitt.edu" <nakajima.erica@medschool.pitt.edu>,"rws9@pitt.edu" <rws9@pitt.edu>,"Van Houten, Bennett" <vanhoutenb@upmc.edu>,"tkensler@pitt.edu" <tkensler@pitt.edu>,"liyang@pitt.edu" <liyang@pitt.edu>,"Fang, Qingming" <fangq@upmc.edu>,"Thompson, Lola" <thompsonla3@upmc.edu>,"George, Lisa (UPCI)" <georgel@upmc.edu>
Cc: "saumen@pitt.edu" <saumen@pitt.edu>,"freerad@pitt.edu" <freerad@pitt.edu>,"Singh, Shivendra" <singhs@upmc.edu>,"jsk5@pitt.edu" <jsk5@pitt.edu>

Dear Li, Erica and Qingming,

As the winners of the Clinical Science Poster competition that was held during this year's UPCI retreat, Dr. Nancy Davidson invites you to participate in our UPCI seminar series. You are each invited to deliver a 15 minute oral presentation at noon on 25th September 2012.

Dr. Davidson will introduce you personally. I suggest we use the following order:

12.00-12.15
1st place: Li Yang et al. “Modulation of estrogen metabolism and estrogen depurinating DNA adducts via
activation of Nrf2 signaling by sulforaphane in breast epithelial cells” (senior author Dr. Thomas
Kensler)

12.20-12.35
2nd place: Erica Nakajima et al. “Diverse metabolite consumption by head and neck squamous cell carcinoma cells”
(senior author Dr. Ben Van Houten)

12.40-12.55
3rd place: Qingming Fang et al. “Complex formation regulates the stability and degradation of PolB and XRCC1” (senior author Dr. Robert Sobol)

I am copying Dr. Freeman because I note that the Department of Pharmacology and Chemical Biology is well represented, to say the least, in these winning posters.

Please save this date for your seminar presentation.

Sincerely,

Chris Bakkenist

Invited review Records:

From: "Jan McIver" <jan.mciver@la-press.com>

Subject: Your Completed Peer Review

Date: Mon, July 9, 2012 7:00 pm

To: liyang@pitt.edu

Dear Dr liyang

On behalf of the Editor in Chief as well as the authors, I would like to thank you for completing your review for Combining mTOR Inhibitors With Chemotherapy and Other Targeted Therapies in Advanced Breast Cancer: Rationale, Clinical Experience, and Future Directions.

The effort you have put into this is most appreciated by us and will be of great value to the authors.

Completing the peer reviewer survey takes less than one minute of your time. Your responses will be anonymous.

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Regards,
Jan

Libertas Academica
la-press.com
(+64-9) 476-3930

From: "Jan McIver" <jan.mciver@la-press.com>

Subject: You Are Invited to Review a Paper [10071]

Date: Tue, June 26, 2012 10:47 pm

To: liyang@pitt.edu

Dear Dr liyang,

I would like to invite you to undertake a peer review of "Combining mTOR Inhibitors With Chemotherapy and Other Targeted Therapies in Advanced Breast Cancer: Rationale, Clinical Experience, and Future Directions", a manuscript submitted by Dr Yardley to Breast Cancer: Basic and Clinical Research.

If you are able to do this I need to receive your review comments by 09 July 2012. Please go to http://la-press.com/review.php?l=YDoXDA7oClKulYZAnBdPA6YS71575 to accept or decline to undertake this review. Please also read the peer review guidelines in this email. The guidelines may be different to those of other journals.

Please confirm your willingness to undertake this review as soon as possible using

the link given above. If you have any questions please email me for assistance.

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4. Click "View Files" to read the manuscript files.
5. Click "Review" to write your review comments.

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Regards,
Jan McIver

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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: Your Completed Peer Review

Date: Thu, May 3, 2012 7:48 pm

To: liyang@pitt.edu

Dear Dr liyang

On behalf of the Editor in Chief as well as the authors, I would like to thank you for completing your review for Insulin-like growth factor 1gene polymorphism and breast cancer risk among Arab Omani women: A case-control study.

The effort you have put into this is most appreciated by us and will be of great value to the authors.

Completing the peer reviewer survey takes less than one minute of your time. Your responses will be anonymous and will be used to enhance our services.

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Regards,
Jan

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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: You Are Invited to Review a Paper [9784]

Date: Thu, April 19, 2012 8:01 pm

To: liyang@pitt.edu

Dear Dr liyang,

I would like to invite you to undertake a peer review of "Insulin-like growth factor 1gene polymorphism and breast cancer risk among Arab Omani women: A case-control study", a manuscript submitted by Professor Al-moundhri to Breast Cancer: Basic and Clinical Research.

If you are able to do this I need to receive your review comments by 04 May 2012. Please go to http://la-press.com/review.php?l=YDoXDA7oClKulYZAnBdPA6YS71575 to accept or decline to undertake this review. Please also read the peer review guidelines in this email. The guidelines may be different to those of other journals.

Please confirm your willingness to undertake this review as soon as possible using the link given above. If you have any questions please email me for assistance.

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Regards,
Jan McIver

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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: Your Completed Peer Review

Date: Wed, April 4, 2012 1:37 pm

To: liyang@pitt.edu

Dear Dr liyang

On behalf of the Editor in Chief as well as the authors, I would like to thank you for completing your review for Circulating immune complex levels are associated with disease severity and seasonality in children with malaria from Mali.

The effort you have put into this is most appreciated by us and will be of great value to the authors.

Completing the peer reviewer survey takes less than one minute of your time. Your responses will be anonymous and will be used to enhance our services.

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Regards,
Jan

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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: You Are Invited to Review a Paper [9624]

Date: Wed, March 21, 2012 6:20 pm

To: liyang@pitt.edu

Dear Dr liyang,

I would like to invite you to undertake a peer review of "Circulating immune complex levels are associated with disease severity and seasonality in children with malaria from Mali", a manuscript submitted by Dr Bolaji N. Thomas to Biomarker Insights.

If you are able to do this I need to receive your review comments by 05 April 2012. Please go to http://la-press.com/review.php?l=YDoXDA7oClKulYZAnBdPA6YS71575 to accept or decline to undertake this review. Please also read the peer review guidelines in this email. The guidelines may be different to those of other journals.

Please confirm your willingness to undertake this review as soon as possible using the link given above. If you have any questions please email me for assistance.

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4. Click "View Files" to read the manuscript files.
5. Click "Review" to write your review comments.

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Regards,
Jan McIver

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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: Your Completed Peer Review

Date: Mon, March 5, 2012 3:49 pm

To: liyang@pitt.edu

Dear Dr liyang

On behalf of the Editor in Chief as well as the authors, I would like to thank you for completing your review for The prognostic value of suPAR compared to other inflammatory markers in patients with severe sepsis.

The effort you have put into this is most appreciated by us and will be of great value to the authors.

Completing the peer reviewer survey takes less than one minute of your time. Your responses will be anonymous and will be used to enhance our services.

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Regards,
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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: Your Completed Peer Review

Date: Mon, March 5, 2012 3:49 pm

To: liyang@pitt.edu

Dear Dr liyang

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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: You Are Invited to Review a Paper [9460]

Date: Thu, February 23, 2012 4:55 pm

To: liyang@pitt.edu

Dear Dr liyang,

I would like to invite you to undertake a peer review of "The prognostic value of suPAR compared to other inflammatory markers in patients with severe sepsis", a manuscript submitted by Mrs Gustafsson to Biomarker Insights.

If you are able to do this I need to receive your review comments by 09 March 2012. Please go to http://www.la-press.com/review.php?l=YDoXDA7oClKulYZAnBdPA6YS71575 to accept or decline to undertake this review. Please also read the peer review guidelines in this email. The guidelines may be different to those of other journals.

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experience for authors.

Regards,
Jan McIver

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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: Your Completed Peer Review

Date: Tue, January 10, 2012 12:31 am

To: liyang@pitt.edu

Dear Dr liyang

On behalf of the Editor in Chief as well as the authors, I would like to thank you for completing your review for Evaluation of ischemia-modified albumin and C-reactive protein in type 2 diabetics with and without ketosis.

The effort you have put into this is most appreciated by us and will be of great value to the authors.

Completing the peer reviewer survey takes less than one minute of your time. Your responses will be anonymous and will be used to enhance our services.

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From: "Jan Mciver" <jan.mciver@la-press.com>

Subject: You are invited to review a paper [9060]

Date: Tue, December 20, 2011 8:59 pm

To: liyang@pitt.edu

Dear Dr liyang,

I would like to invite you to undertake the peer-review of "Evaluation of ischemia-modified albumin and C-reactive protein in type 2 diabetics with and without ketosis". This paper has been submitted by Dr Ma to Biomarker Insights.

If you are able to do this I need to receive your review comments by 09 January 2012.

Please go to http://www.la-press.com/review.php?l=YDoXDA7oClKulYZAnBdPA6YS71575 to accept or decline to undertake this review.

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Regards,
Jan

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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: Your Completed Peer Review

Date: Wed, December 7, 2011 3:45 pm

To: liyang@pitt.edu

Dear Dr liyang

On behalf of the Editor in Chief as well as the authors, I would like to thank you for completing your review for Safety profile of a dietary supplement containing 1,3-dimethylamylamine: a 10-week intervention study.

The effort you have put into this is most appreciated by us and will be of great value to the authors.

Completing the peer reviewer survey takes less than one minute of your time. Your responses will be anonymous and will be used to enhance our services.

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Regards,
Jan

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From: "Jan Mciver" <jan.mciver@la-press.com>

Subject: You are invited to review a paper [8885]

Date: Thu, November 24, 2011 4:58 pm

To: liyang@pitt.edu

Dear Dr liyang,

I would like to invite you to undertake the peer-review of "Safety profile of a dietary supplement containing 1,3-dimethylamylamine: a 10-week intervention study". This paper has been submitted by Dr Bloomer to Nutrition and Metabolic Insights.

If you are able to do this I need to receive your review comments by 07 December 2011.

Please go to http://www.la-press.com/review.php?l=YDoXDA7oClKulYZAnBdPA6YS71575 to accept or decline to undertake this review.

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Regards,
Jan

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From: "Jan McIver" <jan.mciver@la-press.com>

Subject: Your Completed Peer Review

Date: Tue, October 18, 2011 10:20 pm

To: liyang@pitt.edu

Dear Dr liyang

On behalf of the Editor in Chief as well as the authors, I would like to thank you for completing your review for Sustainable long-term delivery microRNA with polylysine nanoparticles for inhibition of breast cancer invasion.

The effort you have put into this is most appreciated by us and will be of great value to the authors.

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From: "Jan Mciver" <jan.mciver@la-press.com>

Subject: You are invited to review a paper [8513]

Date: Mon, October 3, 2011 5:36 pm

To: liyang@pitt.edu

Dear Dr liyang,

I would like to invite you to undertake the peer-review of "Sustainable long-term delivery microRNA with polylysine nanoparticles for inhibition of breast cancer invasion". This paper has been submitted by Dr Jin to Breast Cancer: Basic and Clinical Research .

If you are able to do this I need to receive your review comments by 18 October 2011.

Please go to http://www.la-press.com/review.php?l=YDoXDA7oClKulYZAnBdPA6YS71575 to accept or decline to undertake this review.

How to use My LA to undertake this peer review:
1. Click on the link above as soon as possible after receiving this email.
2. Click on "View Files" to access the abstract and check that you have the expertise to peer-review the paper.
3. Click on "Agree to Review" or "Decline to Review" the manuscript
4. Click "View Files" to read the manuscript files.
5. Click "Review" to write your review comments.

NB: Peer review comments can only be accepted online via My LA. We do not accept downloaded manuscript files that you have annotated or modified in any way.

Please confirm your willingness to undertake this review as soon as possible. If you have any questions please email me for assistance. If you do not want to receive further invitations to review submitted papers, please click here:
http://www.la-press.com/disable_peer_review.php?fp=zoom993&u=liyang2

Instructions for peer reviewers are available here:

http://la-press.com/t.php?i=prguidance

Once you've downloaded the manuscript please ensure you are not in a conflict of interest. Conflict of interest rules are available here: http://la-press.com/t.php?i=reviewers. If you are in a conflict of interest inform me immediately.

You may not have been aware of Breast Cancer: Basic and Clinical Research , which is a new journal. To learn more about it go to http://la-press.com/t.php?i=journallist.

Libertas is a publisher of electronic open and restricted access journals in the scientific, technical and medical areas. We combine conventional production and editorial principles with purpose-developed systems to deliver a fast, high quality experience for authors.

Regards,
Jan

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From: carcinogenesis.editorialoffice@oup.com
Subject: Thank you for submitting your review of manuscript ID CARCIN-2011-00715.R1 for Carcinogenesis
Date: Mon, October 31, 2011 11:25 am
To: liyang@pitt.edu

31-Oct-2011

Dear Ms Yang

Thank you for reviewing manuscript CARCIN-2011-00715.R1 entitled "Catechol-O-methyltransferase-mediated metabolism of 4-hydroxyestradiol inhibits the growth of human renal cancer cells through the apoptotic pathway" for Carcinogenesis.

On behalf of the Editors of Carcinogenesis, we appreciate the voluntary contribution that each reviewer gives to the Journal. We thank you for your participation in the online review process and hope that we may call upon you again to review future manuscripts.

Yours sincerely
Dr Thomas Kensler
Editor
Carcinogenesis

From: carcinogenesis.editorialoffice@oup.com

Subject: Carcinogenesis MS - CARCIN-2011-00715

Date: Tue, August 30, 2011 1:41 pm

To: liyang@pitt.edu

30-Aug-2011

Dear Li:

A manuscript entitled "Catechol-O-methyltransferase-mediated metabolism of 4-hydroxyestradiol inhibits the growth of human renal cancer cells through the apoptotic pathway," with Dr Yuichiro Tanaka as corresponding author, has been submitted to Carcinogenesis, and we are writing to ask whether you could assess it for us. The abstract is shown below. We appreciate that there are many demands on your time, but would greatly value your evaluation of this article.

Could you please let us know whether you would be willing to review this paper for Carcinogenesis, bearing in mind that we would hope to receive the assessment within 2-3 weeks? If you are unable to review this manuscript, we would be grateful for any suggestions for suitable alternative referees, including senior members of your laboratory.

To record your reply automatically, click on the appropriate link below. Details of how to view the manuscript and submit your review will be e-mailed to you as soon as you have agreed.

Agreed: http://mc.manuscriptcentral.com/carcin?URL_MASK=CQyQyR5cFmCRRqGn5T8j

Declined: http://mc.manuscriptcentral.com/carcin?URL_MASK=stXKd5K6D9FJnHdfQ8PF

Alternatively, please reply by return e-mail to carcinogenesis.editorialoffice@oup.com

within 24 hours if at all possible, so that we can continue to provide a timely review process for the research community.

Yours sincerely

Dr Thomas Kensler
Editor
Carcinogenesis

Catechol-O-methyltransferase-mediated metabolism of 4-hydroxyestradiol inhibits the growth of human renal cancer cells through the apoptotic pathway - CARCIN-2011-00715
Corresponding Author: Dr Yuichiro Tanaka
Contributing Authors: Chang, Inik; Liu, Jan; Majid, Shahana; Saini, Sharanjot; Zaman, Mohd; Yamamura, Soichiro; Shahryari, Varahram; Chiyomaru, Takeshi; Deng, Guoren; Dahiya, Rajvir; Tanaka, Yuichiro

Abstract

Long-term exposure to estrogen and its metabolites may play an important role in renal cell carcinogenesis. Catechol-O-methyltransferase (COMT) participates in the estrogen metabolism pathway by neutralizing toxic substances. Although reduced COMT activity has been suggested to be a risk factor for estrogen-associated cancers, no studies have investigated the biological significance of COMT in the pathogenesis of human renal cell cancers (RCC). We initially found that COMT levels are significantly decreased in human RCC tissues and cells suggesting it plays a suppressive role in tumor development. However, transient over-expression of COMT has no functional effect on RCC cell lines. In contrast, when cells over-expressing COMT are treated with its substrate 4-hydroxyestradiol (4-OHE₂), growth is inhibited by apoptotic cell death. We also found that COMT over-expression combined with 4-OHE₂ induces up-regulation of growth arrest- and DNA damage-inducible protein α (GADD45α). We further show that down-regulation of GADD45α by a siRNA-mediated approach inhibits cell death, indicating the essential role of GADD45α in the underlying mechanism of COMT action in response to 4-OHE₂. Finally, 4-methoxyestradiol (4-ME) fully reproduces the anti-proliferative function of COMT with 4-OHE₂ prevents RCC cell proliferation by enhancing apoptosis, and that GADD45α plays a critical role in the COMT-mediated inhibition of RCC.

From: "Intel ISEF Grand Awards Judge Chair" <judging@societyforscience.org>

Subject: ISEF Category Judge Information

Date: Tue, April 24, 2012 6:52 am

To: "Dr. Li Yang" <liyang@pitt.edu>

Thank you for agreeing to serve as a Grand Awards judge at the Intel International Science & Engineering Fair 2012. Please allow this email to serve as a reminder of your commitment as well as general information about the logistics of Tuesday, May 15 and Wednesday, May 16.

SCHEDULE:
Please remember that the time commitment for Grand Awards judging is from Tuesday, May 15, through the evening of Wednesday, May 16. We need you to register in the David L. Lawrence Convention Center no later than 5:00 pm on Tuesday.

Once registered, you should check-in at your category room, pick-up a category ribbon and then spend the time prior to 5:30 p.m. reviewing the projects within your category on the exhibit hall floor. The exhibit hall opens at noon with no finalists present. At 5:30 p.m. all judges will be asked to go back to their respective category rooms for a buffet dinner and brief orientation by the category co-chairs. There will then be a Welcome and training session for all judges from 6:30 - 8:00 p.m. in Hall A. Following this session, the co-chairs will meet their judges in the exhibit hall within their category area to distribute the specific judging interview schedules for the next morning.

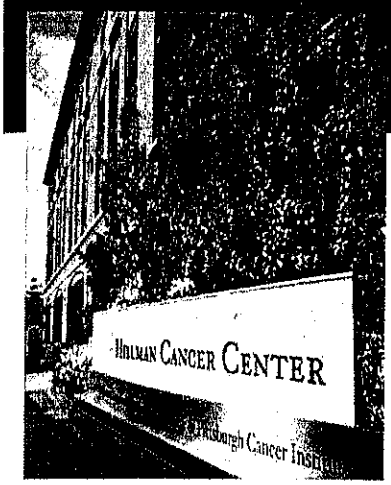
On Wednesday, the Exhibit Hall will be open to judges at 7:00 a.m. and all judges are asked to report to their category rooms by 8:30 for final attendance verification and to receive their score cards. Judging and post-interview category deliberations will occupy all of Wednesday. The detailed schedule for your judging as well as the Judging Guide that explains the complete process is available on the SSP site at http://www.societyforscience.org/page.aspx?pid=290

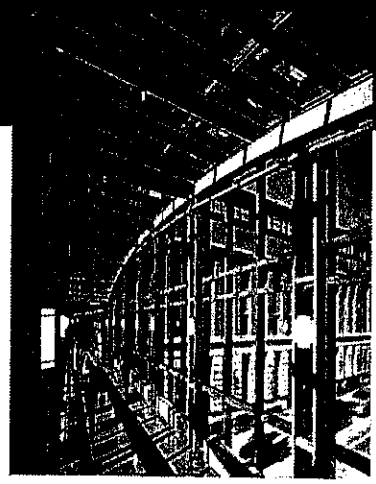
PARKING:
Parking has been arranged for judges and volunteers on Tuesday and Wednesday at the Grand Street Parking Lot between 11th and 12th Streets and Penn and Liberty. A map is available at the website address above. Those that park will need to ask their category co-chair for a chaser card to provide when exiting the garage each day.

MEALS:
All meals will be provided from dinner Tuesday, breakfast-lunch-dinner Wednesday. These will be in the judge`s meeting area.

QUESTIONS:
Send all inquiries to: Judging@societyforscience.org
Thank you again for your commitment to science education and young scientists. We look forward to working with you.

Sincerely,
Chuck






24th Annual UPCI Scientific Retreat

POSTER ABSTRACTS

June 21-22, 2012

University of Pittsburgh at Greensburg

UPMC LIFE CHANGING MEDICINE

Breast Cancer
Translational Science
Poster No. 33.

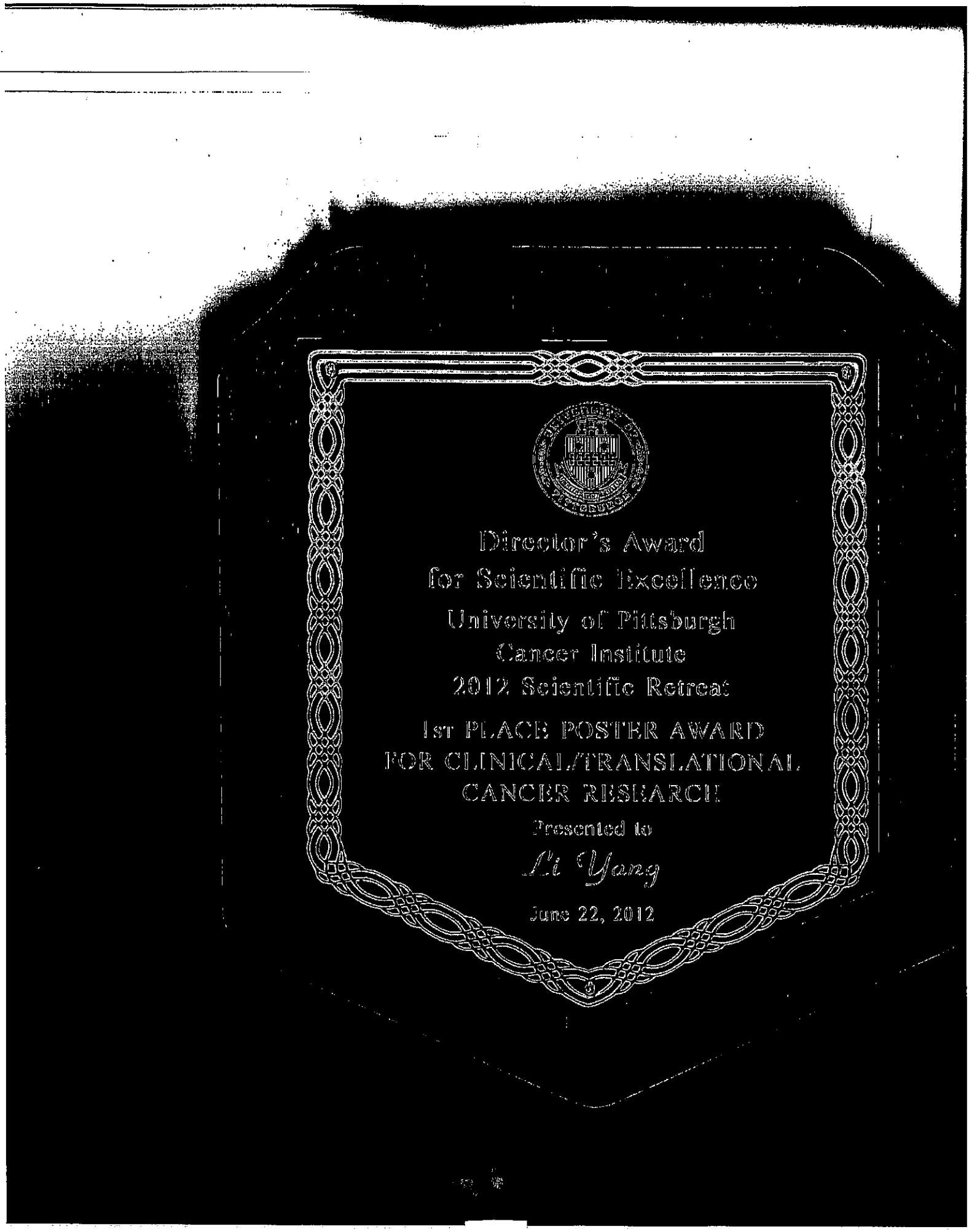
Modulation of estrogen metabolism and estrogen depurinating DNA adducts via activation of Nrf2 signaling by sulforaphane in human breast epithelial cells

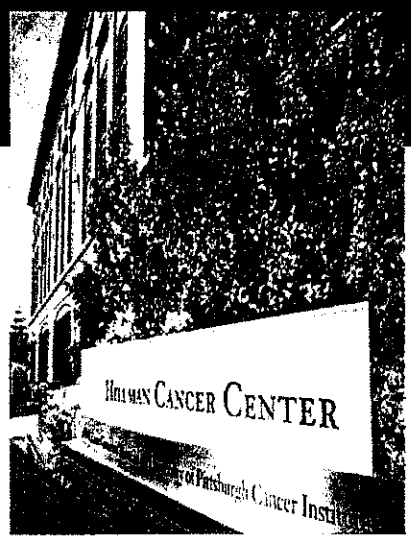
Li Yang(1), Muhammad Zahid(2), Eleanor G. Rogan(2,3), Ercole L. Cavalieri(3), Thomas W. Kensler(1*)

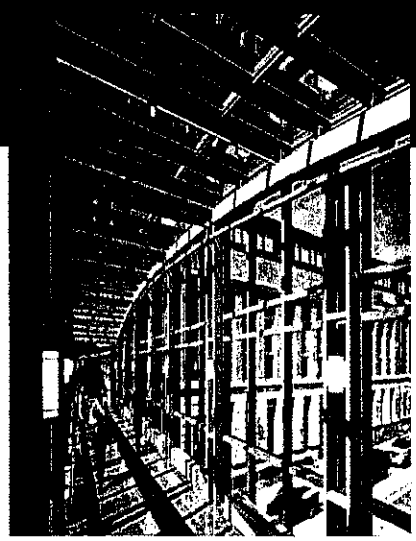
1)Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15213; 2) Environmental, Agricultural and Occupational Health, College of Public Health, and 3)Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68198.

Sulforaphane (SFN) is a potent inducer of detoxification enzymes such as NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-transferase (GST) via the Kelch-like ECH-associated protein 1 (Keap1) - Nuclear Factor- E2-related factor (Nrf2) signaling pathway. NOQ1 reduces the carcinogenic estrogen metabolite, Catechol Estrogen-3,4-Quinone (CE-3,4-Q), to catechols while GSTs detoxify it through nucleophilic addition. CE-3,4-Q can bind with DNA to form depurinating DNA adducts, leading to DNA damage via an estrogen receptor (ER) independent pathway. Thus, SFN, a bioavailable phytochemical found in young broccoli plants, may be an ideal chemoprevention agent to block estrogen-mediated carcinogenesis. For this study we used the ER-negative, nontumorigenic human breast epithelial MCF10A cell line. MCF10A cells were treated with either vehicle or SFN (10 μM) and E2 or 4-OHE2. NQO1 was up-regulated at the mRNA (~2fold), protein (~3fold) and activity levels (~3fold) by SFN treatment. Estrogen metabolites and depurinating DNA adducts in the cell culture medium were partially purified by solid phase extraction and then analyzed by HPLC- ESI-MS/MS. . Following E2 treatment, the depurinated adducts 4-OHE1/2-1-N3Ade and 4-OHE 1/2-1-N7Gua were significantly lower in SFN treated cells compared to vehicle (0.03±0.01 versus 0.07±0.02 pmole/106cell, p=0.0294); 4-OHE1/2- glutathione conjugates were significantly higher following SFN treatment (1.54±0.37 versus 0.83±0.19 pmole/106cell, p=0.0015) as were 4-OCH3E1/2 (5.36 ± 0.16 versus 1.81±0.20pmole/106cell,p<0.0001) levels. Following treatment with the proximate metabolite 4-OHE2 , 4-OHE1/2-1-N3Ade and 4-OHE 1/2-1-N7Gua were again significantly lower in SFN treated cells compared to vehicle (0.59±0.11 versus 1.42±0.16 pmole/106cell, p=0.0028) while 4-OHE1/2-glutathione-conjugates (4.44±0.52 versus 0.87±0.03 pmole/106cell,p=0.0001) and 4-OCH3E1/2 levels were significantly higher (195.00±12.33 versus 58.05±1.77pmole/106cell, p<.00001). Follow-up studies are examining the effects of genetic activation of Nrf2 signaling though disruption of Keap1 as well as targeted silencing of key metabolic genes. In conclusion, SFN can modulate estrogen metabolism leading to diminished formation of estrogen-DNA adducts.

Supported by DOD BCRP Postdoctoral Fellowship103928.


Director's Award
for Scientific Excellence
University of Pittsburgh
Cancer Institute
2012 Scientific Retreat
1st PLACE POSTER AWARD
FOR CLINICAL/TRANSLATIONAL
CANCER RESEARCH
Presented to
Li Yang
June 22, 2012






Annual UPCI Scientific Retreat Satellite Conference

POSTER ABSTRACTS

June 20, 2012

University of Pittsburgh at Greensburg

Cancer Epidemiology, Prevention and Control
Translational Science
Abstract No. 17.

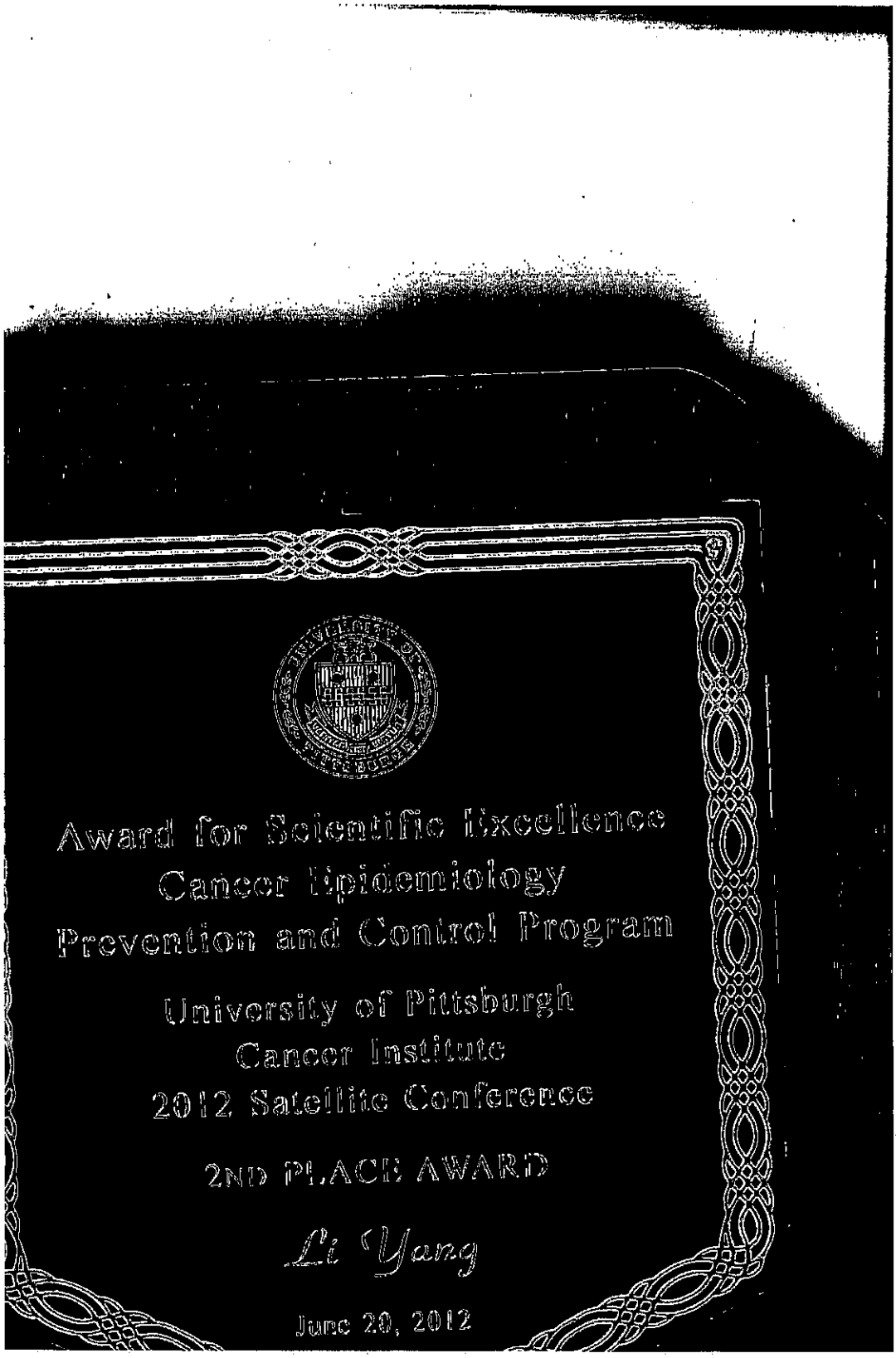
Modulation of estrogen metabolism and estrogen depurinating DNA adducts via activation of Nrf2 signaling by sulforaphane in human breast epithelial cells

Li Yang (1), Muhammad Zahid (2), Eleanor G. Rogan (2,3), Ercole L. Cavalieri (3), Thomas W. Kensler (1*)

1) Department of Pharmacology & Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh, PA, 15213; 2) Environmental, Agricultural and Occupational Health, College of Public Health, and 3) Eppley Institute for Research in Cancer and Allied Diseases, University of Nebraska Medical Center, Omaha, NE 68198

Sulforaphane (SFN) is a potent inducer of detoxification enzymes such as NAD(P)H:quinone oxidoreductase (NQO1) and glutathione-S-transferase (GST) via the Kelch-like ECH-associated protein 1 (Keap1) - Nuclear Factor- E2-related factor (Nrf2) signaling pathway. NOQ1 reduces the carcinogenic estrogen metabolite, Catechol Estrogen-3,4-Quinone (CE-3,4-Q), to catechols while GSTs detoxify it through nucleophilic addition. CE-3,4-Q can bind with DNA to form depurinating DNA adducts, leading to DNA damage via an estrogen receptor (ER) independent pathway. Thus, SFN, a bioavailable phytochemical found in young broccoli plants, may be an ideal chemoprevention agent to block estrogen-mediated carcinogenesis. For this study we used the ER-negative, nontumorigenic human breast epithelial MCF10A cell line. MCF10A cells were treated with either vehicle or SFN (10 μM) and E2 or 4-OHE2. NQO1 was up-regulated at the mRNA (~2fold), protein (~3fold) and activity levels (~3fold) by SFN treatment. Estrogen metabolites and depurinating DNA adducts in the cell culture medium were partially purified by solid phase extraction and then analyzed by HPLC- ESI-MS/MS. Following E2 treatment, the depurinated adducts [redacted] SFN treated cells compared to vehicle ([redacted] versus 0.07±0.02 pmole/106cell, [redacted]); 4-OHE1/2- glutathione conjugates were significantly higher following SFN treatment (1.54±0.37 [redacted] versus 0.83±0.10 pmole/106cell, p=0.0025) as were 4-OCH3E1/2 (5.36±0.16 versus 1.81±0.[redacted] pmole/106cell, p≤0.0001) levels. Following treatment with the proximate metabolite 4-OHE2 , 4-OHE1/2-1-N3Ade and 4-OHE 1/2-1-N7Gua were again significantly lower in SFN treated cells compared to vehicle (0.59±0.11 versus 1.42±0.16 [redacted] while 4-OHE1/2-glutathione-conjugates ([redacted] versus 0.07±0.03 pmole/106cell, p=0.0021) and 4-OCH3E1/2 levels were significantly higher (195.00±12.33 versus 56.66±4.77 pmole/106cell, p<.00001). Follow-up studies are examining the effects of genetic activation of Nrf2 signaling though disruption of Keap1 as well as targeted silencing of key metabolic genes. In conclusion, SFN can modulate estrogen metabolism leading to diminished formation of estrogen-DNA adducts.

Supported by DOD BCRP Postdoctoral Fellowship 103928.


Award for Scientific Excellence
Cancer Epidemiology
Prevention and Control Program
University of Pittsburgh
Cancer Institute
2012 Satellite Conference
2ND PLACE AWARD
Li Yang
June 20, 2012