Application of Bioinformatics for the Functional Genomics Analysis of Prostate Cancer Therapy

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Abstract

Prostate cancer initially responds and regresses in response to androgen depletion therapy, but most human prostate cancers will eventually recur, and re-grow as an androgen independent tumor. Once these tumors become hormone refractory, they usually are incurable leading to death for the patient. Little is known about the molecular details of how prostate cancer cells regress following androgen ablation and which genes are involved in the androgen independent growth following the development of resistance to therapy. Such knowledge would reveal putative drug targets useful in the rational therapeutic design to prevent therapy resistance and control androgen independent growth. The application of genome scale technologies have permitted new insights into the molecular mechanisms associated with these processes. Specifically, we have applied functional genomics using high density cDNA microarray analysis for parallel gene expression analysis of prostate cancer in an experimental xenograft system during androgen withdrawal therapy, and following therapy resistance. The large amount of expression data generated posed a formidable bioinformatics challenge. A novel template based gene clustering algorithm was developed and applied to the data to discover the genes that respond to androgen ablation. The data show restoration of expression of androgen dependent genes in the recurrent tumors and other signaling genes. Together, the discovered genes appear to be involved in prostate cancer cell growth and therapy resistance in this system. We have also developed and applied tissue microarray (TMA) technology for high throughput molecular analysis of hundreds to thousands of clinical specimens simultaneously. TMA analysis was used for rapid clinical translation of candidate genes discovered by cDNA microarray analysis to determine their clinical utility as diagnostic, prognostic, and therapeutic targets. Finally, we have developed a bioinformatic approach to combine pharmacogenomic data on the efficacy and specificity of various drugs to target the discovered prostate cancer growth associated candidate genes in an attempt to improve current therapeutics.

Curriculum Vitae

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Date of Birth: July 24th, 1967

Nationality: Canadian

Education and Research Appointments

09/87- 06/91	**Bachelor of Science**	University of Toronto, Dep of Pharmacology, Toxicology Specialist Program (Honours) Toronto, Canada.
09/91-12/94	**Master of Science**	University of Toronto, Department of Laboratory Medicine & Pathobiology Samuel Lunenfeld Research Institute
12/94-07/98	**Doctor of Philosophy**	University of Toronto, Department of Laboratory Medicine & Pathobiology Samuel Lunenfeld Research Institute Mount Sinai Hospital, Toronto, Canada.
09/98-07/01	**Postdoctoral Fellowship**	National Institutes of Health, National Human Genome Research Institute Bethesda MD, USA. Mount Sinai Hospital, Toronto, Canada.

Honors and Awards:

1991	University of Toronto Life Sciences Award
1993/94	Farber Award, Graduate Department of Pathology, University of Toronto
1996	The Ellen Epstein Rykov Memorial Prize, Faculty of Post-Graduate Medicine, University of Toronto
1996	The Starr Medal, Faculty of Post-Graduate Medicine, University of Toronto
1997	University of Toronto Open Doctoral Fellowship
1998	National Cancer Institute of Canada Travel Fellowship
1998	First Place PhD Poster Award Department of Pathology, University of Toronto
1998-2001	National Institutes of Health Fellowship (Fogarty Visiting Fellow Award)
1999	National Human Research Institute Award for Outstanding Presentation
2000	NIH Fellows Award for Research Excellence (FARE 2000)
2000	AACR/AFLAC Young Investigator & Scholar Award

Teaching Experience:

09/94 – 05/95	Mentorship and technical training of undergraduate students in 4th year laboratory research project. University of Toronto
1996 & 1997	Teaching Assistant, University of Toronto Department of Medical Genetics and Microbiology, Course: Genetics for 2nd Year Medical Students Supervising Professor: Dr. Andrew Becker
1997	Lecturer; Short Series on Northen Blotting Techniques, Michner Institute of Technology of Toronto

NIH Research Trainees

Name	**Position**	**Duration**	**Current Possition**
Tiffany D Miles	Summer Student	06/99-08/99	Ph.D Carnegie Mellon Univ.
Mana Ogholikhan	Summer Student	06/00-08/99	M.D. Georgetown University
Elizabeth Hyman	Howard Hues Fellow	09/00-06-01	M.D. UCLA at Irvine

Professional Activities

Ongoing Extramural Collaborations:

1. Dr. Thomas Pretlow , Case Western University: Functional analysis of hormonal therapy

response in the CWR22 human prostate cancer xenograft model.

2. Dr. Leland Chung, University of Virginia: Identification of metastasis associated genes differentially expressed between the LNCap cell line and the LNCap derivitive C4-2.
3. Dr. Guido Sauter and Dr. Dr. Peter Schraml, Institute of Pathology, University of Basel, Switzerland: Discovery of genes involved in the carcinogenesis of human bladder cancer.
4. Dr. Lukas Bubendorf, and Dr. Guido Sauter University of Basel, Switzerland: Tissue microarray analysis of cDNA microarray candidate genes for protein profiling of various stages of clinical prostate cancer.
5. Dr. Shiv Srivastava, Center for Prostate Disease Research: Androgen receptor regulated genes. Plans to investigate the promoters of candidate genes for androgen regulation.
6. Dr. Hallgeir Rui sand Marja Nevalainen USUH Bethesda MD: Experiments to determine the role of differentially expressed candidate genes on androgen receptor signaling and prostate cancer growth.
7. Dr. Jane Trepel, Medicine Branch, NCI, NIH: Collaboration to test new drugs currently in clinical trials for the molecular mechanisms by which they inhibit the growth of advanced prostate cancer.
8. Drs. William Isaacs and John Isaacs at the Johns Hopkins University: Analysis of gene expression of clinical tumors and orthotopic prostate cancer xenografts.

Patent application: Genes related to development of refractory prostate cancer.
Inventors: Spyro Mousses, Lukas Bubendorf, Olli-P Kallioniemi

Society Membership: Associate member of American Association of Cancer Research

Reviewer for Grants: Hong Kong Research Grants Council

Reviewer for Journal: Clinical Cancer Research

International Lectures by Invitation:

04/1999 "Molecular mechanisms underlying endocrine therapy failure in human prostate cancer analysed by DNA and tissue microarrays" Minisymposium at AACR 90th Annual Meeting Philadelphia, PA, USA

05/1999 "cDNA microarray technology development and applications"
Plenary Session: Genetic Toxicology Association Annual Meeting, Newark DW.

10/1999 "Gene Expression Profiling by cDNA Microarrays of Hormone Therapy Failure in Human Prostate Cancer" Symposium at National Human Genome Research

Institute Annual Retreat, Arlie, VA, USA

03/2000 "Biological basis of hormone refractory prostate cancer" Plenary Session at European Congress of Andrology by the European Academy of Andrology L'Aquila Italy,

04/2000 "Hormone Therapy Failure in Prostate Cancer Analyzed by Gene Expression Profiling using cDNA and Tissue microarrays..." Minisymposium at AACR 91st Annual Meeting San Fransico CA, USA

06/2000 "cDNA microarray analysis ..." Symposium at XVI European Association of Cancer Research Meeting in Halkidiki, Greece

08/2000 "cDNA microarray analysis of prostate cancer progression" Guest Speaker for Department of Urology Seminar Series, University of Vigrinia, USA

09/2000 "Functional Genomics of Hormone Therapy Response and Resistance in Prostate Cancer" Plenary presentation in Genomics Session of the Austrian Society of Genetics and Genetic Engineering Annual Meeting, Innsbruck, Austria

11/2000 "Applications of Bioinformatics" Plenary presentation at Biological Research Information Center symposium: Pohang, Korea

02/2001 "cDNA microarray analysis of hormone therapy response and resistance in prostate cancer" Plenary Symposium at XVIth Testis Workshop, Newport Beach CA, USA

SCIENTIFIC PUBLICATIONS

Research Articles (peer-reviewed)

1. **Mousses, S.**, Ozcelik, H., Lee, P., Malkin, D., Bull, S., Andrulis, I.L. (1995) Two variants of the CIP1/WAF1 gene occur together and are associated with human cancer. *Hum Mol Gen* 4:1089-92.
2. Ozcelik, H., **Mousses, S.** and Andrulis, I.L. (1995) Low levels of expression of an inhibitor of cyclin-dependent kinases (CIP1/WAF1) in primary breast carcinomas with p53 mutations. *Clinical Cancer Research* 1:907-912.
3. Mousses, S., McAuley, L., Bell, R., Kandel, R., Andrulis, I.L. (1996) Molecular and immunohistochemical identification of p53 alterations in bone and soft tissue sarcomas. *Modern Pathology* 9:1-6.
4. Bubendorf L., Kolmer M., Kononen J., Koivisto P., **Mousses S.**, Chen Y., Mahlamaki E., Schraml P., Moch H., Willi N., Elkahloun A.G., Pretlow T.G., Gasser T.C., Mihatsch M.J., Sauter G., Kallioniemi O-P. (1999) Hormone therapy failure in human prostate cancer: analysis by complementary DNA and tissue microarrays. *J Natl Cancer Inst* 91(20):1758-

64

5. **Mousses, S.**, Gokgoz, N., Ozcelik, H., Wunder, J.S., Bell, R.S., Andrulis, I.L. (1999) p53 Missense Mutations, but not Truncation Mutations, are Associated with Low Levels of *p21* mRNA Expression in Primary Human Sarcomas. *Submitted Manuscript Oncogene*
6. **Mousses, S.**, Bubendorf, L., Kononen, J., Chen, Y., Bittner, M.L., Koivisto, P., Trepel, J., Kim, JW., Raffeld, M., Pretlow, T., Goldberger, N., Cornelison, R., Wagner, U., Hostetter, G., Sauter, G., Kallioniemi, O-P. (2000) Functional and translational genomics analysis of hormone therapy for prostate cancer reveal candidate diagnostic and drug targets *Submitted Manuscript PNAS*
7. **Mousses S.**, Kim J.W., Chen Y., Bittner M., Kallioniemi O-P., Trepel J (2000) cDNA microarray analysis prostate cancer cell inhibition using a novel HDAC inhibitor reveals a TGF-beta mediated mechanism *Manuscript in preparation*
8. **Mousses, S.,** Kim, J. W., Kim, J. S., Sausville, E. A., Chen, Y., Nakanishi, O., Nakajima, H., Kallioniemi, O.-P., Kim, S.-J., and Trepel J. B. Global gene expression patterns in response to inhibition of histone deacetylase identify TGF-beta signaling as a novel histone deacetylase target. *Manuscript in preparation*

Reviews& Book Chapters:

1. **Mousses, S.**, Pressacco, J., Ozcelik, H., (1996) Guarding the genome: A review of the p53 tumour suppressor gene. *University of Toronto Medical Journal* Vol. 73(3) pp.148-156
2. **Mousses, S.** (1999) Gearing for Automatic Genomics Automation: Genome and Functional Analyses, Methods in Microbiology (Vol. 28) edited by A.G. Craig and J.D. Hoheisel *Trends in Genetics* Vol.15(11) p. 473
3. **Mousses, S.**, Bubendorf, L., Kononen, J., Bittner, M., Chen, Y., Willi, N., Pretlow, T., Sauter, G., Kallioniemi, O-P. (2000) Biological basis of hormone refractory human prostate cancer: Gene Discovery by Parallele mRNA Expression Analysis and Translation to Clinical High Throughput Molecular Pathology *Int J Androl.* Vol. 23 Supp.1.
4. **Mousses, S.,** Bittner ML, Chen Y, Dougherty ER., Baxevanis A., Meltzer PS., and Trent JM. (2000) Gene Expression Analysis by cDNA Microarrays (Book Chapter in Press) in "*Differential Gene Expression: A practical approach*", published by Oxford University Press, eds. F.J. Livesey and S.P. Hunt.
5. **Mousses, S.**,Microarray Techology. (Book Chapter in Press) in "*Cancer Research: an Encyclopedic Reference*" published by Springer Press, ed. M. Schwab
6. **Mousses, S.,** et al. Prostate Cancer Progression, review article in preparation for *Endocrine-Related Cancer*

Abstracts/Conference Presentations:

1. **Mousses, S.**, McAuley, L., Wunder, J.S., Noble-Topham, S.E., Bell, R.S., and Andrulis, I.L., "Evaluation of p53 mutations and MDR1 levels in Osteosarcoma." *40th Annual Meeting, Orthopaedic Research Society, Feb. 21-24, 1994. New Oreleans, Louisiana, USA.*
2. Slingerland, J., Shaw, P., Catsavelos, C., **Mousses, S.**, Ozcelik, H., and Andrulis, I.L., "G1/S phase cyclin expression and activity in primary breast carcinoma." *Cold Spring Harbor Laboratories, 59th Symposium on Quantitative Biology, The Cell Cycle, May 18-22 1994. Cold Spring Harbor, NY, USA*
3. Ozcelik, H., **Mousses, S.** and Andrulis, I.L. "Low mRNA levels of p53-regulated inhibitor of cyclin-dependent kinase (CIP1/WAF1) in breast cancer." *Cold Spring Harbor Laboratory, 59th symposium on quantitative biology, Molecular Genetics of Cancer, June 1-8, 1994, Cold Spring Harbor, NY, USA*
4. **Mousses, S.**, Ozcelik, H., Bell, R.S., and Andrulis, I.L. "Analysis of the involvement of p21(CIP1/WAF1) gene expression in human sarcomas." *41st Annual Meeting, Orthopaedic Research Society, February 13-16, 1995, Orlando, Florida. USA*
5. Gokgoz, N., **Mousses, S.**, Wunder, J.S., Bell, R.S., and Andrulis, I.L. "Characterisation of p53 Mutations in Osteosarcoma" *Connective Tissue Oncology Society 2nd Annual Scientific Meeting, October 10-13, 1996.*
6. To, M.D., **Mousses, S.**, Scherer, S.W., Beatty, B.G., Tsui, L-C., and Andrulis I.L. "Identification of potential proto-oncogenes and tumor suppressor genes in breast cancer by differential display." *Cold Spring Harbor Laboratory, Cancer Genetics & Tumor Suppressor Genes, August 14-18, 1996, Cold Spring Harbor, New York, USA.*
7. **Mousses, S.**, Ozcelik, H., To, M.D., and Andrulis, I.L. "Germ-line variants of p21(CIP1/WAF1) and differences in allele specific expression" *Cold Spring Harbor Laboratory, Cancer Genetics & Tumor Suppressor Genes, August 14-18, 1996, Cold Spring Harbor, New York, USA.*
8. **Mousses, S.**, Ozcelik, H., To, M.D., Wunder, J.S., Bell, R.S., and Andrulis, I.L. "Post-transcriptional and p53-independent regulation of p21CIP1/WAF1 expression." *88th Annual Meeting of the American Association for Cancer Research. Volume 38 (1997) of the Proceedings of the American Association for Cancer Research. San Diego, California, USA. April 12-16, 1997.*
9. **Mousses, S.**, Gokgoz, N., Ozcelik, H., Wunder, J.S., Bell, R.S., and Andrulis I.L. "p53 mutations and transcriptional regulation of downstream genes" *2nd Annual Workshop on Methods and Applications of DNA Microarray Technology, Tucson, AZ, USA. Jan 11-13, 1998.*
10. Gokgoz, N., **Mousses, S.**, Wunder, J., Bell, R.S., and Andrulis, I.L. "Molecular analysis of the p53 gene in human osteosarcoma" *89th Annual Meeting of the American Association*

for Cancer Research. Volume 39 (1998) of the Proceedings of the American Association for Cancer Research. New Orleans, LA, USA. March 28-April 1, 1998.

11. Gokgoz, N., **Mousses, S.**, Wunder, J., Bell, R.S. and Andrulis, I.L. "Mutations in the p53 gene are an early event in human osteosarcoma progression." *90th Annual Meeting of the AACR. Volume 40 (1999) of the Proceedings of the American Association for Cancer Research. Philidelphia, Pennsylvania, April 10-14, 1999.*
12. **Mousses, S.**, Bubendorf, L., Kononen, J., Kolmer, M., Elkahloun, A., Koivisto, P., Pretlow, T., Schraml, P., Gasser, T.C., Sauter, G., Kallioniemi, O-P., "Molecular mechanisms underlying endocrine therapy failure in human prostate cancer analysed by DNA and tissue microarrays" 90th *Annual Meeting of the American Association for Cancer Research. Volume 40 (1999) of the Proceedings of the American Association for Cancer Research. Philidelphia, Pennsylvania, USA. April 10-14, 1999. (ORAL PRESENTATION)*
13. **Mousses, S.**, Bubendorf, L., Kononen, J., Kolmer, M., Kallioniemi, O-P. "Molecular Mechanisms Underlying Endocrine Therapy Failure In Human Prostate Cancer Analyzed by DNA and Tissue Microarrays" *Annual Meeting of the American Urological Association. Huston, Texas, April 1999.*
14. **Mousses, S.**, Bubendorf, L., Kononen, J., Bittner, M., Chen, Y., Kolmer, M., Elkahloun, A., Koivisto, P., Pretlow, T., Schraml, P., Sauter, G., Kallioniemi, O-P. Identification of genes involved in hormone-independent prostate cancer by cDNA Microarrays, followed by in vivo analysis of selected genes using tissue microarray analysis. *The Nature Genetics Microarray Meeting in Scottsdale, Arizona Sept. 22-25, 1999.*
15. **Mousses, S.**, Bubendorf, L., Kononen, J., Bittner, M., Chen, Y., P., Pretlow, T., Koivisto, P., Sauter, G., Kallioniemi, O-P. cDNA microarray analysis and multi-template gene clustering revealed a temporal pattern of gene expression that explains the initial regression and eventual resistance that occurs during hormonal therapy of human prostate cancer. *8th International Workshop on Chromosomes in Solid Tumors January 30-February 1, 2000 in Tucson, Arizona(ORAL PRESENTATION)*
16. **Mousses, S.**, Bubendorf, L., Kononen, J., Bittner, M., Willi, N., Gasser, T.C., Sauter, G., Kallioniemi, O-P. "Gene Expression Profiles Associated with Androgen Deprivation Response and Therapy Failure in Human Prostate Cancer: High-Throughput cDNA and Tissue Microarray Studies" *Annual Meeting of the American Urological Association. Atlanta, Georgia. April 29- May 4, 2000.*
17. **Mousses, S.**, Bubendorf, L., Kononen, J., Bittner, M., Chen, Y., Willi, N., Pretlow, T., Sauter, G., Kallioniemi, O-P. "Hormone Therapy Failure in Prostate Cancer Analyzed by Gene Expression Profiling using cDNA and Tissue microarrays: From Gene Discovery to Molecular Pathology" . *Volume 41 (2000) of the Proceedings of the American Association*

for Cancer Research, San Francisco, CA April 1-5, 2000. (ORAL PRESENTATION)