SCIENTIFIC REPORT
2007-2008

DEPARTMENT OF
CANCER PREVENTION

INSTITUTE FOR CANCER RESEARCH

NORWEGIAN RADIUM HOSPITAL

Centre for Cancer Biomedicine

RIKSHOSPITALET

TABLE OF CONTENTS

SUMMARY

Department of Cancer Prevention was established May 2006 and is one of seven departments at the Institute of Cancer Research.

Our focus is secondary and tertiary cancer prevention, aiming to identify, develop and validate new biomarkers with high sensitivity and specificity. These can be applied to early diagnosis, risk assessment as well as prediction of disease course and treatment response. Colorectal cancer and the many clinical challenges yet unresolved are the crux of the department's research activities. We are increasing our activity also on urological cancers.

Understanding molecular mechanisms underlying human tumour development is essential to improve the diagnosis and treatment of the cancer patient. To gain knowledge of the complex dynamics of these abnormal processes our department combines large-scale and detailed biology research using *in vitro* models and human samples.

Our department is a member of the CoE – Centre for cancer biomedicine, University of Oslo, and we cooperate with the other groups within the Centre's major scientific programs.

During the two-year period 2007-2008 we have increased the staff from 24 to 38 people. The department includes two research groups, "molecular genetics" (Lothe) and "molecular cell biology" (Rivedal). A project group of "genome biology" was established August 2007 (Skotheim). In this period, we have published 23 original research papers, 4 other scientific publications, filed 3 patent applications, and four M.Sc. degrees are defended.

Professor

Ragnhild A. Lothe

Head of Department of Cancer Prevention

SCIENTIFIC ACTIVITY

The scientific achievements in the two-year period 2007-2008 are summarized on pages 4-7 and additional relevant activities are described on pages 8-10.

CENTRES OF EXCELLENCE, UNIVERSITY OF OSLO

Our department is a member of **CoE in Research - Centre for Cancer Biomedicine**, that was established in 2007 and consists of 7 research groups (Stenmark, Sandvig, Smeland, Liestøl, Olsnes, Lothe, and Danielsen). Harald Stenmark is Director and Ragnhild A. Lothe is Vice-director for the Centre.

CoE in Innovation – Centre for cancer stem cell was established in 2007 and consists of 10 research groups, including Lothe lab, as well as industrial partners.

PAPERS

Ahlquist T , Bottillo I, Danielsen SA , Meling GI, Rognum TO, Lind GE, Dallapiccola B, Lothe RA. RAS signaling in Colorectal Carcinomas through alterations of RAS, RAF, NF1 and/or RASSF1A. **Neoplasia**, 10: 680-686 (+2pp following 686), 2008.

Ahlquist T, Lind GE, Skotheim RI, Meling GI, Vatn M, Hoff GE, Rognum TO, Thiis-Evensen E, Lothe RA. Gene methylation profiles of normal mucosa, and benign and malignant colorectal tumors identify early onset markers. **Mol. Cancer** (BMC), 7:94, 2008.

Alagaratnam S, Hardy JR, Lothe RA, Skotheim RI, Byrne JA. TPD52, a candidate gene from genomic studies, is overexpressed in testicular germ cell tumours. **Mol. Cell. Endocrinol.**, 2008 [Epub ahead of print].

Almstrup K, Leffers H, Lothe RA, Skakkebæk N, Sonne SB, Nielsen JE, Rajpert-De Meyts E, Skotheim RI. Gene expression patterns in testicular carcinoma in situ - re-analysis of joint microarray data. **Int. J. Androl**., 30:292-303, 2007.

Barsnes H, Eidhammer I, Cruciani V, Mikalsen SO. Protease-dependent fractional mass and peptide properties. **Eur. J. Mass. Spectrom.**, 14:311-7, 2008.

Barsnes H, Mikalsen SO, Eidhammer I. Blind search for post-translational modifications and amino acid substitutions using peptide mass fingerprints from two proteases. **BMC Res. Notes**, 1:130, 2008.

Bottillo I, Ahlquist T, Brekke HR, Danielsen SA, van den Berg E, Mertens F, Lothe RA, Dallapiccola B. Germline and somatic *NF1* mutations in sporadic and NF1-associated malignant peripheral nerve sheath tumors. **J. Pathol.,** 2008 Nov 10 ahead of print

Cruciani V and Mikalsen SO. Evolutionary selection pressure and family relationships among connexin genes. **Biol. Chem**., 388(3): 253-64, 2007.

Danielsen SA, Lind GE, Bjørnslett M, Meling GI, Rognum TO, Heim S, Lothe RA. Novel mutations of the suppressor gene *PTEN* in colorectal carcinomas stratified by microsatellite instability- and *TP53* mutation-status. **Hum. Mut.**, 29: E252-62, 2008.

Eidhammer I, Barsnes H, Mikalsen SO. MassSorter: peptide mass fingerprinting data analysis. **Methods Mol. Biol.**, 484:345-59, 2008.

Haugen MH, Flatmark K, Mikalsen SO, Mælandsmo GM. The metastasis-associated protein S100A4 exists in several charged variants suggesting the presence of posttranslational modifications. **BMC Cancer**, 8:172, 2008.

Hoei-Hansen C, Kraggerud SM, Abeler VM, Kærn J, Chris Lau YF, Rajpert-De Meyts E, Lothe RA. Ovarian dysgerminomas are characterized by frequent KIT mutations and abundant expression of pluripotency markers. **Mol. Cancer**, 6:12, 2007.

Kilpinen S, Autio R, Ojala K, Iljin K, Bucher E, Sara H, Pisto T, Saarela M, Skotheim RI, Björkman M, Mpindi JP, Haapa-Paananen S, Vainio P, Edgren H, Wolf M, Astola J, Nees M, Hautaniemi S, Kallioniemi O. Systematic bioinformatic analysis of expression levels of 17,330 human genes across 9,783 samples from 175 types of healthy and pathological tissues. **Genome biol.**, 9: R139, 2008.

Kleivi K, Lind GE, Diep CB, Meling GI, Brandal LT, Nesland JM, Myklebost O, Rognum TO, Giercksky KE, Skotheim RI, Lothe RA. Gene expression profiles of primary colorectal carcinomas, liver metastases and carcinomatoses. **Mol. Cancer**, 6:2, 2007.

Leithe E and Rivedal E. Ubiquitination of gap junction proteins. **J. Membr. Biol.**, 217: 43-51, 2007.

Lind GE, Skotheim RI, Lothe RA. The epigenome of testicular germ cell tumors. **APMIS**, 115:1147-1160, 2007.

Lind GE, Ahlquist T, LotheRA. DNA hypermethylation of MAL – a promising diagnostic biomarker for colorectal tumors. **Gastroenterology**, 132: 1631-2, 2007.

Lind GE, Ahlquist T, Kolberg M, Berg M, Eknæs M, Alonso MA, Kallionemi A, Meling GI, Thiis-Evensen E, Rognum TO, Lothe RA. Hypermethylated MAL – a silent marker of early colon tumorigenesis. **J. Translational Medicine** (BMC "Highly accessed"), 6:13, 2008.

Oldenburg J, Kraggerud SM, Cvancarova M, Lothe RA, Fosså SD. Cisplatin-induced long-term hearing impairment is associated with specific glutathione-s-transferase genotypes in testicular cancer survivors. **J. Clin. Oncol.**, 25: 708-14, 2007.

Oldenburg J, Kraggerud SM, Brydøy M, Cvancarova M, Lothe RA, Fosså SD. Long term neurotoxicities in testicular cancer survivors are associated with polymorphisms in glutathione-s-transferase-P1 and-M1. **J. Translational Medicine** (BMC), 5:70 (pp), 2007.

Ribeiro FR, Skotheim RI, Henrique R, and Teixeira MR. Book chapter 30: Array-based comparative genomic hybridization in prostate cancer: research and clinical applications. In: Methods of cancer diagnosis, therapy and prognosis (Hayat MA, Ed.), volume II, Springer Publishers, USA, 2008.

Rivedal E, Leithe E. The benzene metabolite trans,trans-muconaldehyde blocks gap junction intercellular communication by cross-linking connexin43. **Toxicol. Appl. Pharmacol.**, 232:463-8, 2008.

Røyrvik E, Ahlquist T, Rognes T, Lothe RA. „Slip Slidin' Away: A duodecennial review of targeted genes in mismatch repair deficient colorectal cancer" invited review, CRO-**Critical Reviews in Oncogenesis**, 13: 229-257, 2007. Front cover.

Sirnes S, Leithe E, Rivedal E. The detergent resistance of Connexin43 is lost upon TPA or EGF treatment and is an early step in gap junction endocytosis. **Biochem. Biophys. Res. Commun.**, 373:597-601, 2008.

Skotheim RI and Nees M. Alternative splicing in cancer: noise, functional, or systematic? **Int. J. Biochem. Cell Biol**, 39: 1432-49, 2007.

Wu Q, Lothe RA, Ahlquist T, Silins I, Tropé CG, Micci F, Nesland JM, Suo Z, Lind GE. DNA methylation profiling of ovarian carcinomas and their *in vitro* models identifies *HOXA9*, *HOXB5*, *SCGB3A1*, and *CRABP1* as novel targets. **Mol. Cancer**, 6:45 (pp.10), 2007 (BMC: Flagged as "Highly accessed")

Wu Q, Lind GE, Aasheim HC, Silins I, Tropé CG, Nesland JM, Lothe RA, Suo Z. The ephrin receptor Bs (EPHBs) promoters are unmethylated in colorectal- and ovarian- carcinomas. **Epigenetics**, 2 (4): (pp), 2007.

ACADEMIC THESES

Ellen C. Røyrvik, M.Sc., Dept. Molecular Biosciences, Faculty of Mathematics and Natural Sciences, U of Oslo, 2007, "Slip Slidin'away: Mutations in colorectal cancer with microsatellite instability". Main supervisor: Ragnhild A. Lothe, co-supervisor: Terje Ahlquist.

Hilde Honne, M.Sc., Dept. Molecular Biosciences, Faculty of Mathematics and Natural Sciences, U of Oslo, 2008, "Identification of novel epigenetic biomarkers in colorectal cancer" Main supervisor: Guro E. Lind, co-supervisor: Ragnhild A. Lothe.

Jarle Bruun, M.Sc., Dept. Molecular Biosciences, Faculty of Mathematics and Natural Sciences, U of Oslo, 2008, "Effect of Connexin 43 transfection on growth characteristics of the human colon adenocarcinoma cell line HT29" Main supervisor: Edward Leithe, co-supervisor: Edgar Rivedal.

Marthe Eken, M.Sc., Dept. Molecular Biosciences, Faculty of Mathematics and Natural Sciences, U of Oslo, 2008, "Identification of cancer specific transcripts: With emphasis on the hunt for fusion genes in colorectal cancer". Main supervisor: Rolf I. Skotheim, co-supervisor: Ragnhild A. Lothe.

PATENTS

May 2007, PA200700601. Markers for tumour development in the aero-digestive system.

June 2007, PA200700930 / EP07111167.8 (EU). Fusion gene microarray.

Dec 2008, EP08172043.5. A method for identification of RNA transcript variants.

AWARDS / HONORS

2007

May

Edgar Rivedal is elected national member of the Scientific Council of IARC - International agency for research on cancer, Lyon. (WHO). The Council consists of 20 country representatives.

May

Professor Ragnhild A. Lothe is elected as member of the Norwegian Society of Letters and Science.

June

Senior research fellow Rolf I. Skotheim is appointed guest professor at CoE - Centre of Genome-scale Biology, VTT and U Helsinki, Finland.

June

Professor Ragnhild A. Lothe awarded Kong Olav the Vth cancer research prize

Oct.

Postdoc Guro E. Lind and co-workers receive Medinnova's Idea Prize (TTO for Oslo University hospital).

2008

Oct.

Prof. Rolf I. Skotheim and co-workers is 1st runner up for Medinnova's Idea prize.

Oct.

Prof. Ragnhild A. Lothe assigned professor at The Medical Faculty, University of Oslo.

Nov.

Prof. Rolf I. Skotheim receives Young Investigator Award, Annual Meeting for Oncologists, Norway.

SCIENTIFIC CONSULTING

Ragnhild A. Lothe:

    Member of research council of RR-HF and U of Oslo

    Member of CCC (comprehensive cancer centre)-council, RR-HF

    Head of grant committee for basic research, Norwegian Cancer Society, second period: 2004 - 2007

    Head of the Research Council of the Norwegian Cancer Society, Election period: 2008-2009.

    Member of the Forensic medicine committee, Justis department, Election period: 2006-2008

    Head of the board, Norwegian Centre Molecular Medicine, U of Oslo, EMBL partnership.

    Board member, Oslo Cancer Cluster. (2008-)

    Grant (Linnaeus grant) evaluation for Swedish Research Council. 2008.

    Member of group leader search committee for Institute for Molecular Medicine Finland – FIMM, EMBL partnership. Helsinki, FIN. 2008.

Edgar Rivedal:

    Member of Norwegian Scientific Committee for Food Safety (VKM) (2005-)

    Member of the Scientific Council for International Agency of Research on Cancer (IARC), Lyon, France (2007-)

    Member of a working group appointed by the Ministry of Defence to evaluate the health risk of occupational radar radiation (2007).

    Personnel Safety Representative at Institute for Cancer Research (2007-).

    Censor for Master theses: Tove Igeland (August 2008), Siv Basmoen (November 2008), NTNU, Trondheim.

Rolf I. Skotheim:

    Opponent for Ph.D. thesis of Stian Knappskog, Medical Faculty., U of Bergen, March 2007.

    Opponent for Ph.D. thesis of Gerd Moe-Behrens, Medical faculty, U of Oslo, Feb. 2008.

    Opponent for Ph.D. thesis of Sine Brask Sonne. Faculty of Health Sciences, U of Copenhagen, Oct. 2008.

    Member of grant committee for basic research, Norwegian Cancer Society (2008-).

    Head of expert evaluation board for Assistant Professorship, Oslo University College.

Guro E.Lind:

    Member of the Scientific Council, Norwegian Cancer Society. (2008-)

    Member of working group for generating common standard operating procedures for the new research building for Inst Cancer Research (2008-), Oslo University hospital.

Matthias Kolberg:

    Chairman of the Oslo branch of the Norwegian Biochemical Society (NBS), 2007.

2007

Jan   EMBO conference series, "pre-mRNA processing and disease". Cortina d'Ampezzo, Italia, 2007.

Feb   Oncogenomics, Phoenix, USA.

March   Workshop: Whole Genome and TaqMan Low Density Array, Geilo, NOR 2007.

March   AACR Annual Meeting, Los Angeles, USA.

March   "A cancer study using the AB1700 system, challenges from the laboratory and QC interpretations" Applied Biosystems user meeting, Geilo, NOR.

March   "Experimental strategy for new theragnostics" Folkehelseinstituttet, NOR.

April   AACR Annual Meeting, LA, USA.

May   "Risk assessment of pesticides", Regulatory toxicology, U of Oslo, NOR.

May   "Integrated genome and transcriptome analyses – examples from testicular cancer", Rikshosp., Oslo, NOR.

June   "Genome biology of cancer", VTT Medical Biotechnology, Turku, FIN.

June   "Epigenetic biomarkers for early diagnosis of colorectal cancer" Meddinova Idea prize, Oslo, NOR. Receiver Guro E. Lind.

July   "Inhibition of gap junction intercellular communication by benzene metabolites. Role in hematotoxicity and leukemia?" International Congress of Toxicology, Montreal, Canada. Invited speaker

Aug.   International gap junction conference, Helsingør, DEN

Sept.   "Application of IST for biomarker and outlier analyses" VTT, Medical biotechnology conference, Nauvo, FIN. Invited speaker

Oct.   Evidence based toxicology, Cernobbio, Italy

Oct.   "Epigenetic markers in colorectal tumorigenesis". Ahus symposium, colorectal disease, inflammation and cancer. Oslo, NOR. Invited speaker

Nov   "Biomarkers for early detection of colorectal cancer", Onkologisk Forum, Oslo, NOR. "King Olav Vth Prize lecture" Receiver: Ragnhild A.Lothe

Nov.   AACR special conference: "Advances in Colon Cancer Research", Boston, USA.

Dec.   Whole Transcript Expression Meeting, Stansted, UK.

2008

Feb.   AACR Special meeting: Ubiquitin and Cancer, San Diego, USA

Feb.   AACR special conference: "Cancer Stem Cells", LA, USA

March   "Early detection of colorectal cancer." Norwegian State visit to Portugal. Biotechnology seminar, University of Porto, POR. Invited delegate.

March   "Genome biology of human testicular tumourigenesis." European Testis Workshop, Naantali, Finland. Invited speaker.

March   "Screening for colorectal cancer?", Helse og Sosialdirektoratet, Oslo, NOR. Invited speaker.

April   AACR Annual Meeting, San Diego, USA.

May   AACR special conference: "Cancer Epigenetics", Boston, USA.

Sept.   "Mechanisms for Down-Regulation of Gap Junction Intercellular Communication in Cancer." The 14th International Charles Heidelberger Symposium on Cancer Research, Urumqi, China. Invited speaker.

Oct.   "Novel epigenetic biomarkers for early detection of colorectal tumors." Workshop: Translational research-based decision-making in CRC, Taormina, Italy. Invited EACR delegate.

Oct.   International TP53 workshop. Shanghai, China.

Oct.   "Identification of novel biomarkers for early detection of colorectal tumors". Biomarker Discovery Europe 2008, GTCbio, Dublin, Irland. Invited speaker

Nov.   "Nuclear TP53 expression in malignant peripheral nerve sheath tumors is an independent marker of poor survival" Connective Tissue Oncology Society 14th Annual Meeting, London UK.

Nov.	"Cancer specific RNA in search for biomarkers and targets for treatment", Onkologisk Forum, Bergen, NOR. "Young Investigator award lecture 2008". Receiver: Rolf I. Skotheim.
Nov.	AACR special conference: "Targeting the PI3K pathway in cancer", Boston, USA.
Nov.	"Early detection of colorectal cancer", Kunnskapsdepartementet, Oslo, NOR. Invited speaker.
Dec.	"Novel epigenetic biomarkers for early detection of colorectal tumors". Genesis, London, UK. Invited speaker, delegate of Oslo Cancer Cluster.

EDITORIAL ACTIVITY

Ragnhild A. Lothe Ed-in-Chief, Rolf I. Skotheim Managing editor: "Critical Reviews of Oncogenesis- CRO", Begell House, NY publisher. 4 issues per year volume since 2006.

Editorial member, Comprehensive Cancer Centre Scientific Report for 2006-2007.

Referee work among senior staff members for the following journals: APMIS (1), BBA-Biomembranes (1), BMC Bioinformatics (1), BMC Cancer (2), BMC Cell Biology (1), Br. J. Cancer (1), Cancer Lett. (1), Chem. Res. Toxicol. (1), Clin Chem (1), Eur. J. Cell Biol. (1), Genes Chrom. Cancer (3), Gut (1), Int. J. Androl. (4), Int J Cancer (1), J. Pathol. (4), J. Proteome Res. (1), Lancet Oncol. (1), Neoplasia (2).
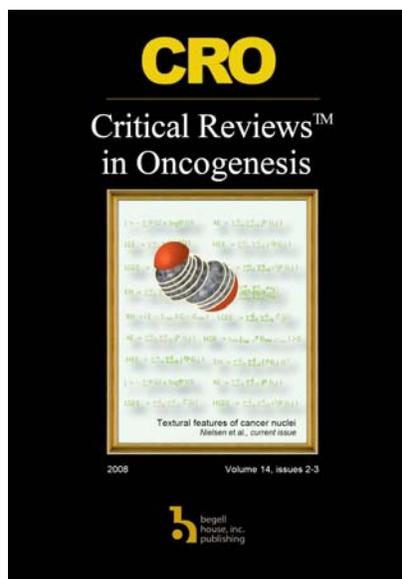
ONGOING PROJECTS

INTERPLAY OF EPIGENETICS AND GENETICS IN DEVELOPMENT OF COLORECTAL CANCER, PROSTATE CANCER AND MALIGNANT PERIPHERAL NERVE SHEATH TUMORS

Novel markers are identified through large-scale approaches and their integrative analyses (see below). Candidates are submitted to detailed analyses at the level of DNA, RNA and protein. This includes the use of sequencing, RT-PCR (e.g. TaqMan-based) and tissue microarrays. New biological information of the developmental cancer process is obtained. Some markers are forwarded for functional studies and some to analyses in larger clinical series for examination of their suitability as early biomarkers or prognostic markers.
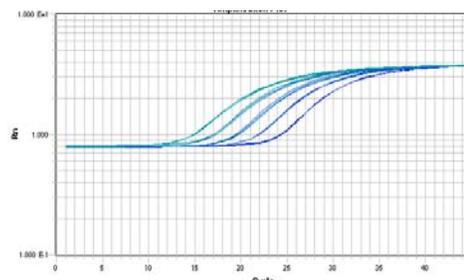
PREDICTIVE AND PROGNOSTIC TRANSCRIPT SIGNATURES OF COLORECTAL CANCER
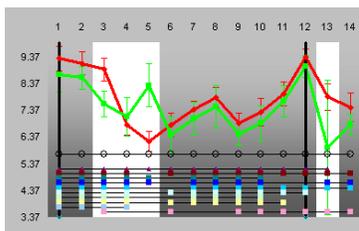
Molecular profiling of clinical consecutive series of primary carcinomas before and after adjuvant therapy enable search for true predictive signatures. Furthermore, a long-term follow up with high quality clinical data allow us to examine the prognostic information among large series of Dukes B and C stages. Both test and validation series are currently investigated by use of Affymetrix exon arrays.

EARLY ONSET COLORECTAL CANCER

In order to identify early somatic changes in development of CRC and potentially identify candidates for genetic predisposition a series of tumors from early onset patients (<50ye) with no known hereditary syndrome is compared with tumors from elderly patients (>70ye). The sample series are matched for sex, tumor location and Dukes stages and are submitted to analyses of high resolution copy number changes (Nimblegen micorarrays) and to transcriptome analyses.
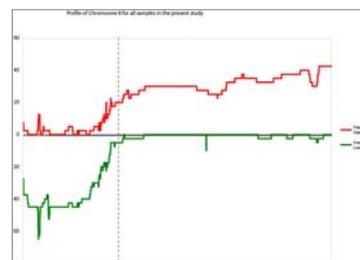
EPIGENOMICS

A strategy combining drug treatment of cell lines with transcriptome analyses of primary tumours identify genome-wide gene targets for methylation (Lind et al., Cell. Oncol. 2006). This has been applied on CRC and novel biomarkers are identified and validated (Lind et al., Gastroenterology 2007; Lind et al., J. Transl. Med. 2008; Ahlquist et al., Mol. Cancer 2008, Lind et al., submitted).

We are currently using this strategy for studies of other cancers. These studies are performed in collaborative settings ensuring optimal biobanks and know-how of the individual cancer types.
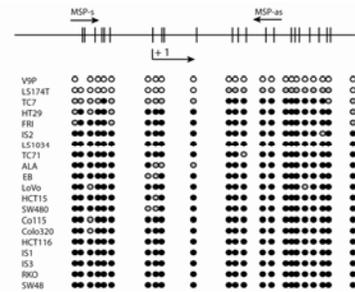
QUALITATIVE TRANSCRIPTOMICS
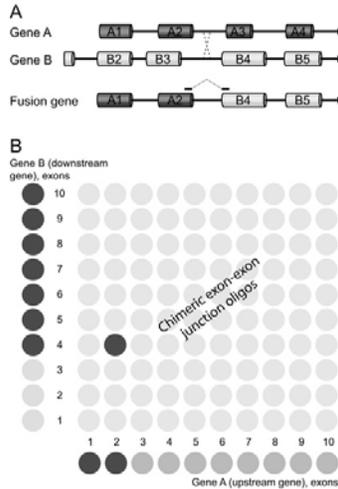
A new microarray-based tool for universal detection of fusion genes has been developed (Skotheim et al., Mol. Cancer 2009). We are in progress to evaluate the diagnostic potential of this tool through blinded analyses of cancer samples obtained from two European diagnostic labs. Furthermore, it will be used in the colorectal cancer research program and for a screen of cell lines across several cancer types.

The Affymetrix exon array platform is established in lab and used for both quantitative and qualitative transcriptomics. The latter may pinpoint potential fusion gene partners, but mostly it is used for identification of splice variants and novel transcripts.

PROTEIN DEGRADATION MECHANISMS

With particular focus on the process of intercellular communication and a protein family of connexins we study protein degradation mechanisms with special emphasis on the role of ubiquitin. The interplay between different post-translational modifications may contribute to explain how these proteins are regulated. With main focus on colorectal cancer a combined genetics, epigenetics and cell biology approaches are ongoing to unravel the potential roles of the various connexin isoforms in development of this disease.
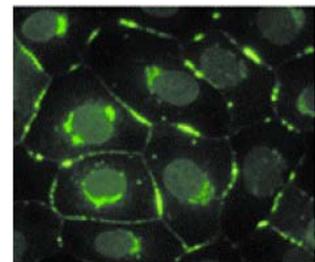
ACTIVE PARTNERS

Prof. Manuel Teixeira lab

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Prof. Peter Andrews lab

University of Sheffield, Great Britain

Prof. Olli Kallioniemi lab

VTT and FIMM, Finland

Prof. Fredrik Mertens lab

University of Lund, Sweden

Prof. Yasufumi Omori

Akita University, Japan

Prof. Bruno Dallapiccola

University of Rome, Rome, Italy

CAST Centre for Cancer Stem Cells
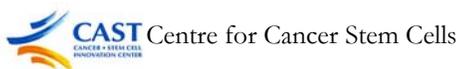
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Eva van den Berg,
Department of Genetics, University Medical Centre Groningen, University of Groningen, The Netherlands

EDUCATION PROGRAMS

SCIENCE

The dept senior staff members educate at bachelor and master level, as well as supervision of Ph.D. and post-doctoral work.

Bachelor course: University of Oslo, MBV3020, Development and molecular genetics.

New Master and PhD course in "cancer biology" agreed with University of Oslo, Faculty of Mathematics and natural Sciences. Run from 2009.

Postdocs: 5 ongoing

Ph.D.: 10 Ongoing: nine associated with Medical Faculty, and one with the Faculty of Mathematics and Natural Sciences, University of Oslo.

M.Sc.: 6 ongoing: Dept. Molecular Biosciences, the Faculty of Mathematics and Natural Sciences, University of Oslo.

LEADERSHIP

Rolf I. Skotheim. Leadership development course, "Essential Leader", Harvard ManageMentor and Case in Point., 12 half-day gatherings 2006-2007.

Guro E. Lind. Advanced Laboratory Management course: "Self and time management", 3 days EMBO course, Germany, 2007.

Advanced leadership skills training course: "Coaching", 3 days EMBO course, Germany.

Ragnhild A. Lothe. Three gatherings autumn 2008 – Department heads at the Institute for Cancer Research. Including team management profiling.

ADMINISTRATIVE

The department policy, in line with the Institutional policy, is to obtain safe environment through sufficient training and education.

Stine A. Danielsen. Course in safety work for administrators and personnel safety representatives, 2007.

Guest scientists at department of Cancer Prevention:

Postdoc Franclim Ribeiro, Oncology Institute, University of Porto, Portugal. 3 months Jan-March 2007, 2 months May-June 2007, 2 months spring 2008

Ph. D student Vera Costa, Oncology Institute, University of Porto, Portugal. 1 month autumn 2006, 2 months spring 2007, 2 months spring 2008.

Department staff visiting foreign laboratories:

Ph.D. student Terje Ahlquist visited Prof. Bruno Dellapiccola laboratory, University of Rome, Italy. April-Mai 2007.

Postdoc Sharmini Alagaratnam visited Prof. Peter Andrews laboratory, University of Sheffield, UK. Feb-March 2008

SOCIAL AND SCIENTIFIC INTERPLAY

ORGANISATION/STAFF

Department of Cancer Prevention was established May 2006 and is headed by Professor Ragnhild A. Lothe. Administrative staff: Economy: Linda U. Mjøen (30 %), Human Resources: Mona Hagen (20 %). Laboratory manager: Guro E. Lind, Responsible for personnel safety: Stine Aske Danielsen, Responsible for fire protection: Solveig Sirnes.

Molecular Genetics

Implementing molecular genetics, epigenetics and molecular pathology methods into the department projects is the responsibility of the „Molecular genetics group" lead by Prof Ragnhild A.Lothe. The P.I. is also responsible for the biobanks and the strategy for the translational research projects.

Molecular Cell biology

Implementing advance microscopy and various cell biology techniques in the different department projects is the responsibility of the „Molecular cell biology group" lead by Dr. Edgar Rivedal

Genome biology

Development of and implementing high throughput technologies and advanced bioinformatics in the various department projects is the responsibility of the "Genome biology project" lead by Prof. Rolf Skotheim.

The department has 32 employees and 6 master students. The staff include 2 administrative personnel, 4 prof./senior research fellows, 6 research associates/postdocs, 13 research assistants/Ph.D. students, 3 medical technologists, 2 technicians (part time), 2 guest scientists (as of Jan. 2009).

Terje Ahlquist – PhD student

Sharmini Alagaratnam – Post doc

Deeqa Ali – Master student

Kim Andresen – PhD student

Anne Cathrine Bakken – Master student

Marianne Berg – PhD student

Jarle Bruun – Research associate

Lina Cekaite – Post doc

Inger Christiansen - Technician

Vera Costa – Guest PhD student

Stine A Danielsen – PhD student

Anne-Lise Edvardsen - Technician

Hans Geir Eiken – Senior researcher

Marthe Eken – PhD student

Mette Eknæs - Medical technologist

Tone Fykerud – Master student

Marianne Guriby – Master student

Mona Hagen - Administrative

Merete Hektoen - Medical technologist

Hilde Honne – Research assistant

Maren Høland – Master student

Ane Kjenseth – PhD student

Matthias Kolberg - Scientist

Sigrid Marie Kraggerud - Scientist

Edward Leithe – Post doc

Guro E Lind – Scientist

Ragnhild A Lothe – Head of Dept

Marianne A Merok – PhD student

Linda Uv Mjøen – Administrative

Franclim Ribeiro – Guest scientist

Edgar Rivedal – Group leader

Rolf I Skotheim – Project leader

Gard Thomassen – PhD Student

Zeremariam Yohannes – Medical technologist

Solveig Sirnes – PhD student

Anita Sveen – PhD student

Hege Marie Vedeld – Master student

Trude H Ågesen – PhD student

FUNDING AND COSTS

Total budget for 2007 and 2008 were 13.0 and 15.9 million NOK, respectively. Internal funding was 5.4 million NOK in 2007 and 5.3 million NOK in 2008. External funding was 7.6 million NOK in 2007 and 10.6 million NOK in 2008.

Internal vs. external funding sources, 2007 and 2008.
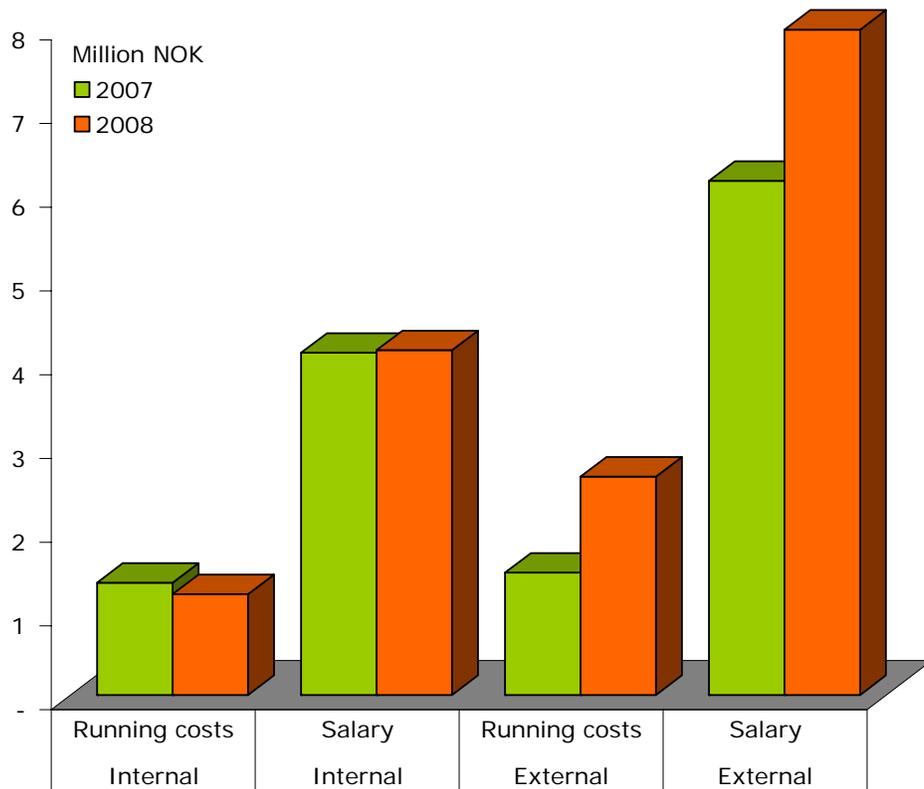
PAPER ABSTRACTS

Ahlquist T , Bottillo I, Danielsen SA , Meling GI, Rognum TO, Lind GE, Dallapiccola B, Lothe RA. **RAS signaling in Colorectal Carcinomas through alterations of RAS, RAF, NF1 and/or RASSF1A**. Neoplasia, 10: 680-686 (+2pp following 686), 2008.

More than half of all colorectal carcinomas are known to exhibit an activated mitogen-activated protein kinase pathway. The NF1 gene, a negative regulator of KRAS, has not previously been examined in a series of colorectal cancer. In the present study, primary colorectal carcinomas stratified according to microsatellite instability status were analyzed. The whole coding region of NF1 was analyzed for mutations using denaturing high-performance liquid chromatography and sequencing, and the copy number alterations of NF1 were examined using multiple ligation-dependent probe amplification and real-time polymerase chain reaction. The mutational hot spots in KRAS and BRAF were sequenced, and promoter hypermethylation status of RASSF1A was assessed with a methylation-specific polymerase chain reaction. One sample had two missense mutations in NF1, whereas nine additional tumors had intronic mutations likely to affect exon splicing. Interestingly, 8 of these 10 tumors were microsatellite-unstable. Four other tumors showed a duplication of NF1. Mutations in KRAS and BRAF were mutually exclusive and were present at 40% and 22%, respectively. RASSF1A was hypermethylated in 31% of the samples. We show that the RAS signaling network is extensively dysregulated in colorectal carcinomas, because more than 70% of the tumors had an alteration in one or more of the four examined components.

Ahlquist T, Lind GE, Skotheim RI, Meling GI, Vatn M, Hoff GE, Rognum TO, Thiis-Evensen E, Lothe RA. **Gene methylation profiles of normal mucosa, and benign and malignant colorectal tumors identify early onset markers**. Mol. Cancer (BMC), 7:94, 2008.

BACKGROUND: Multiple epigenetic and genetic changes have been reported in colorectal tumors, but few of these have clinical impact. This study aims to pinpoint epigenetic markers that can discriminate between non-malignant and malignant tissue from the large bowel, i.e. markers with diagnostic potential. The methylation status of eleven genes (ADAMTS1, CDKN2A, CRABP1, HOXA9, MAL, MGMT, MLH1, NR3C1, PTEN, RUNX3, and SCGB3A1) was determined in 154 tissue samples including normal mucosa, adenomas, and carcinomas of the colorectum. The gene-specific and widespread methylation status among the carcinomas was related to patient gender and age, and microsatellite instability status. Possible CIMP tumors were identified by comparing the methylation profile with microsatellite instability (MSI), BRAF-, KRAS-, and TP53 mutation status. RESULTS: The mean number of methylated genes per sample was 0.4 in normal colon mucosa from tumor-free individuals, 1.2 in mucosa from cancerous bowels, 2.2 in adenomas, and 3.9 in carcinomas. Widespread methylation was found in both adenomas and carcinomas. The promoters of ADAMTS1, MAL, and MGMT were frequently methylated in benign samples as well as in malignant tumors, independent of microsatellite instability. In contrast, normal mucosa samples taken from bowels without tumor were rarely methylated for the same genes. Hypermethylated CRABP1, MLH1, NR3C1, RUNX3, and SCGB3A1 were shown to be identifiers of carcinomas with microsatellite instability. In agreement with the CIMP concept, MSI and mutated BRAF were associated with samples harboring hypermethylation of several target genes. CONCLUSION: Methylated ADAMTS1, MGMT, and MAL are suitable as markers for early tumor detection.

Alagaratnam S, Hardy JR, Lothe RA, Skotheim RI, Byrne JA. **TPD52, a candidate gene from genomic studies, is overexpressed in testicular germ cell tumours**. Mol. Cell. Endocrinol., 2008

Several genomic regions are recurrently over- or underrepresented in testicular germ cell tumours (TGCTs), but only a fraction of their genes change their expression accordingly. Two publications to date have studied DNA copy numbers and associated gene expression changes on a genome-wide level to identify key players in TGCT tumorigenesis. Here, we compare lists of significant genes in these studies, and show that 17 genes are common to both. These include concomitant gain and over-expression of JUB, NRXN3, and TPD52, and loss and under-expression of C11orf70 and CADM1, in addition to 12 overexpressed genes located on

the chromosome arm 12p. We performed immunohistochemical analysis of TPD52 on a tissue microarray, which showed complete absence of TPD52 protein in normal germ cells and most intratubular germ cell neoplasias. TPD52 was expressed in two-thirds of seminomas and embryonal carcinomas, and at intermediate frequencies in the more differentiated non-seminomas.

Almstrup K, Leffers H, Lothe RA, Skakkebæk N, Sonne SB, Nielsen JE, Rajpert-De Meyts E, Skotheim RI. **Improved gene expression signature of testicular carcinoma in situ.**. Int. J. Androl., 30:292-302, 2007.

The carcinoma in situ (CIS) stage is the common precursor of testicular germ cell tumours (TGCTs) that arise in young adults. Within the past decade genome wide gene expression tools have been developed and have greatly advanced the insight into the biology of TGCTs. Two independent data sets on global gene expression in testicular CIS have been previously published. We have merged the two data sets on CIS samples (n = 6) and identified the shared gene expression signature in relation to expression in normal testis. Among the top-20 highest expressed genes, one-third was transcription factors and the list included some 'novel' CIS markers (i.e. DOCK11 and ANXA3). Genes related to biological terms 'nucleic acid binding' and 'translational activity' (e.g. transcription factors and ribosomal proteins, respectively) were consistently and significantly over-represented. Some of the significantly over-expressed genes in CIS cells were selected for validation by RT-PCR (IFI16, DOCK11, and ANXA3), immunohistochemistry (HLXB9), or in situ hybridization (IFI16). High-level analysis utilizing the Ingenuity pathway analysis tool indicated that networks relating to 'gene expression in cancer' and 'embryonic development' were significantly altered and could collectively affect cellular pathways like the WNT signalling cascade, which thus may be disrupted in testicular CIS. The merged CIS data from two different microarray platforms, to our knowledge, provide the most precise CIS gene expression signature to date.

Barsnes H, Eidhammer I, Cruciani V, Mikalsen SO. **Protease-dependent fractional mass and peptide properties**. Eur. J. Mass. Spectrom., 14:311-7, 2008.

Mass spectrometric analyses of peptides mainly rely on cleavage of proteins with proteases that have a defined specificity. The specificities of the proteases imply that there is not a random distribution of amino acids in the peptides. The physico-chemical effects of this distribution have been partly analyzed for tryptic peptides, but to a lesser degree for other proteases. Using all human proteins in Swiss-Prot, the relationships between peptide fractional mass, pI and hydrophobicity were investigated. The distribution of the fractional masses and the average regression lines for the fractional masses were similar, but not identical, for the peptides generated by the proteases trypsin, chymotrypsin and gluC, with the steepest regression line for gluC. The fractional mass regression lines for individual proteins showed up to +/-100 ppm in mass difference from the average regression line and the peptides generated showed protease-dependent properties. We here show that the fractional mass and some other properties of the peptides are dependent on the protease used for generating the peptides. With the increasing accuracy of mass spectrometry instruments it is possible to exploit the information embedded in the fractional mass of unknown peaks in peptide mass fingerprint spectra

Barsnes H, Mikalsen SO, Eidhammer I. **Blind search for post-translational modifications and amino acid substitutions using peptide mass fingerprints from two proteases**. BMC Res. Notes, 1:130, 2008.

BACKGROUND: Mass spectrometric analysis of peptides is an essential part of protein identification and characterization, the latter meaning the identification of modifications and amino acid substitutions. There are two main approaches for characterization: (i) using a predefined set of possible modifications and substitutions or (ii) performing a blind search. The first option is straightforward, but can not detect modifications or substitutions outside the predefined set. A blind search does not have this limitation, and therefore has the potential of detecting both known and unknown modifications and substitutions. Combining the peptide mass fingerprints from two proteases result in overlapping sequence coverage of the protein, thereby offering alternative views of the protein and a novel way of indicating post-translational modifications and amino acid substitutions. RESULTS: We have developed an algorithm and a software tool, MassShiftFinder, that performs a blind search using peptide mass fingerprints from two proteases with different cleavage specificities. The algorithm is based on equal mass shifts for overlapping peptides from the two

proteases used, and can indicate both post-translational modifications and amino acid substitutions. In most cases it is possible to suggest a restricted area within the overlapping peptides where the mass shift can occur. The program is available at http://www.bioinfo.no/software/massShiftFinder. CONCLUSION: Without any prior assumptions on their presence the described algorithm is able to indicate post-translational modifications or amino acid substitutions in MALDI-TOF experiments on identified proteins, and can thereby direct the involved peptides to subsequent TOF-TOF analysis. The algorithm is designed for detailed and low-throughput characterization of single proteins.

Bottillo I, Ahlquist T, Brekke HR, Danielsen SA, van den Berg E, Mertens F, Lothe RA, Dallapiccola B. **Germline and somatic NF1 mutations in sporadic and NF1-associated malignant peripheral nerve sheath tumors**. J. Pathol., 2008 Nov 10 ahead of print

Malignant peripheral nerve sheath tumours (MPNSTs) are a malignancy occurring with increased frequency in patients with neurofibromatosis type 1 (NF1). In contrast to the well-known spectrum of germline NF1 mutations, the information on somatic mutations in MPNSTs is limited. In this study, we screened NF1, KRAS, and BRAF in 47 MPNSTs from patients with (n = 25) and without (n = 22) NF1. In addition, DNA from peripheral blood and cutaneous neurofibroma biopsies from, respectively, 14/25 and 7/25 of the NF1 patients were analysed. Germline NF1 mutations were detected in ten NF1 patients, including three frameshift, three nonsense, one missense, one splicing alteration, and two large deletions. Somatic NF1 mutations were found in 10/25 (40%) NF1-associated MPNSTs, in 3/7 (43%) neurofibromas, and in 9/22 (41%) sporadic MPNSTs. Large genomic copy number changes accounted for 6/10 and 7/13 somatic mutations in NF1-associated and sporadic MPNSTs, respectively. Two NF1-associated and 13 sporadic MPNSTs did not show any NF1 mutation. A major role of the KRAS and BRAF genes was ruled out. The spectrum of germline NF1 mutations in neurofibromatosis patients with MPNST is different from the spectrum of somatic mutations seen in MPNSTs. However, the somatic events share common characteristics with the NF1-related and the sporadic tumours.

Cruciani V and Mikalsen SO. **Evolutionary selection pressure and family relationships among connexin genes**. Biol. Chem., 388(3): 253-64, 2007.

We suggest an extension of connexin orthology relationships across the major vertebrate lineages. We first show that the conserved domains of mammalian connexins (encoding the N-terminus, four transmembrane domains and two extracellular loops) are subjected to a considerably more strict selection pressure than the full-length sequences or the variable domains (the intracellular loop and C-terminal tail). Therefore, the conserved domains are more useful for the study of family relationships over larger evolutionary distances. The conserved domains of connexins were collected from chicken, Xenopus tropicalis, zebrafish, pufferfish, green spotted pufferfish, Ciona intestinalis and Halocynthia pyriformis (two tunicates). A total of 305 connexin sequences were included in this analysis. Phylogenetic trees were constructed, from which the orthologies and the presumed evolutionary relationships between the sequences were deduced. The tunicate connexins studied had the closest, but still distant, relationships to vertebrate connexin 36, 39.2, 43.4, 45 and 47. The main structure in the connexin family known from mammals pre-dates the divergence of bony fishes, but some additional losses and gains of connexin sequences have occurred in the evolutionary lineages of subsequent vertebrates. Thus, the connexin gene family probably originated in the early evolution of chordates, and underwent major restructuring with regard to gene and subfamily structures (including the number of genes in each subfamily) during early vertebrate evolution.

Danielsen SA, Lind GE, Bjørnslett M, Meling GI, Rognum TO, Heim S, Lothe RA. **Novel mutations of the suppressor gene PTEN in colorectal carcinomas stratified by microsatellite instability- and TP53 mutation- status**. Hum. Mut., 29: E252-62, 2008.

PTEN regulates cell homeostasis by inhibiting growth signals transduced through PI3-kinases. The gene is mutated in several cancer types, but so far, only a limited number of mutations have been reported in colorectal cancer. In the present study, direct sequencing was used to analyze the whole coding region and exon-intron boundaries of PTEN in a series of microsatellite stable (n=34) and microsatellite unstable (n=30) colorectal carcinomas with known TP53 mutation status. We detected 21 PTEN mutations in altogether 13 tumors (20%), including 19 mutations in the coding sequence and two in the exon-intron boundaries. Sixteen of these alterations have not been previously reported in colorectal cancer. Furthermore, seven out of the 13

altered tumors harbored more than one mutation, potentially leading to loss of gene function. Finally, all PTEN mutations found were in tumors harboring wild-type TP53. In conclusion, PTEN is mutated in a significant subgroup of colorectal carcinomas, and our findings further extend the previously small spectrum of reported PTEN changes. Additionally, it seems that mutations in PTEN and TP53 are mutually exclusive for this cancer type.

Eidhammer I, Barsnes H, Mikalsen SO. **MassSorter: peptide mass fingerprinting data analysis**. Methods Mol. Biol., 484:345-59, 2008.

MassSorter is a software tool that sorts, systemizes, and analyzes data from peptide mass fingerprinting (PMF) experiments on proteins with known amino acid sequences. Several experiments can be simultaneously analyzed for sequence coverage and posttranslational modifications occurring during sample handling, induced chemical modifications, and unexpected cleavages. Experimental m/z values are compared with m/z values from an in silico digestion, taking modifications into account. Filters can be defined by users for marking autolytic protease peaks and other contaminating peaks. MassSorter functions as a database of all the detected peptides. It includes tools for visualization of the results, such as sequence coverage, accuracy plots, statistics, and 3D models.

Haugen MH, Flatmark K, Mikalsen SO, Mælandsmo GM. **The metastasis-associated protein S100A4 exists in several charged variants suggesting the presence of posttranslational modifications**. BMC Cancer, 8:172, 2008.

BACKGROUND: S100A4 is a metastasis-associated protein which has been linked to multiple cellular events, and has been identified extracellularly, in the cytoplasm and in the nucleus of tumor cells; however, the biological implications of subcellular location are unknown. Associations between a variety of posttranslational protein modifications and altered biological functions of proteins are becoming increasingly evident. Identification and characterization of posttranslationally modified S100A4 variants could thus contribute to elucidating the mechanisms for the many cellular functions that have been reported for this protein, and might eventually lead to the identification of novel drugable targets. METHODS: S100A4 was immuoprecipitated from a panel of in vitro and in vivo sources using a monoclonal antibody and the samples were separated by 2D-PAGE. Gels were analyzed by western blot and silver staining, and subsequently, several of the observed spots were identified as S100A4 by the use of MALDI-TOF and MALDI-TOF/TOF. RESULTS: A characteristic pattern of spots was observed when S100A4 was separated by 2D-PAGE suggesting the presence of at least three charge variants. These charge variants were verified as S100A4 both by western immunoblotting and mass spectrometry, and almost identical patterns were observed in samples from different tissues and subcellular compartments. Interestingly, recombinant S100A4 displayed a similar pattern on 2D-PAGE, but with different quantitative distribution between the observed spots. CONCLUSION: Endogenously expressed S100A4 were shown to exist in several charge variants, which indicates the presence of posttranslational modifications altering the net charge of the protein. The different variants were present in all subcellular compartments and tissues/cell lines examined, suggesting that the described charge variants is a universal phenomenon, and cannot explain the localization of S100A4 in different subcellular compartments. However, the identity of the specific posttranslational modification and its potential contribution to the many reported biological events induced by S100A4, are subject to further studies.

Hoei-Hansen C, Kraggerud SM, Abeler VM, Kærn J, Chris Lau YF, Rajpert-De Meyts E, Lothe RA. **Ovarian dysgerminomas are characterized by frequent KIT mutations and abundant expression of pluripotency markers**. Mol. Cancer, 6:12, 2007.

BACKGROUND: Ovarian germ cell tumours (OGCTs) typically arise in young females and their pathogenesis remains poorly understood. We investigated the origin of malignant OGCTs and underlying molecular events in the development of the various histological subtypes of this neoplasia. RESULTS: We examined in situ expression of stem cell-related (NANOG, OCT-3/4, KIT, AP-2gamma) and germ cell-specific proteins (MAGE-A4, NY-ESO-1, TSPY) using a tissue microarray consisting of 60 OGCT tissue samples and eight ovarian small cell carcinoma samples. Developmental pattern of expression of NANOG, TSPY, NY-ESO-1 and MAGE-A4 was determined in foetal ovaries (gestational weeks 13-40). The molecular genetic part of our study included search for the presence of Y-chromosome material by fluorescence in situ

hybridisation (FISH), and mutational analysis of the KIT oncogene (exon 17, codon 816), which is often mutated in testicular GCTs, in a subset of tumour DNA samples. We detected a high expression of transcription factors related to the embryonic stem cell-like pluripotency and undifferentiated state in OGCTs, but not in small cell carcinomas, supporting the view that the latter do not arise from a germ cell progenitor. Bilateral OGCTs expressed more stem cell markers than unilateral cases. However, KIT was mutated in 5/13 unilateral dysgerminomas, whereas all bilateral dysgerminomas (n = 4) and all other histological types (n = 22) showed a wild type sequence. Furthermore, tissue from five phenotypic female patients harbouring combined dysgerminoma/gonadoblastoma expressed TSPY and contained Y-chromosome material as confirmed by FISH. CONCLUSION: This study provides new data supporting two distinct but overlapping pathways in OGCT development; one involving spontaneous KIT mutation(s) leading to increased survival and proliferation of undifferentiated oogonia, the other related to presence of Y chromosome material and ensuing gonadal dysgenesis in phenotypic females.

Kilpinen S, Autio R, Ojala K, Iljin K, Bucher E, Sara H, Pisto T, Saarela M, Skotheim RI, Björkman M, Mpindi JP, Haapa-Paananen S, Vainio P, Edgren H, Wolf M, Astola J, Nees M, Hautaniemi S, Kallioniemi O. **Systematic bioinformatic analysis of expression levels of 17,330 human genes across 9,783 samples from 175 types of healthy and pathological tissues**. Genome biol., 9: R139, 2008.

Our knowledge on tissue- and disease-specific functions of human genes is rather limited and highly context-specific. Here, we have developed a method for the comparison of mRNA expression levels of most human genes across 9,783 Affymetrix gene expression array experiments representing 43 normal human tissue types, 68 cancer types, and 64 other diseases. This database of gene expression patterns in normal human tissues and pathological conditions covers 113 million datapoints and is available from the GeneSapiens website.

Kleivi K, Lind GE, Diep CB, Meling GI, Brandal LT, Nesland JM, Myklebost O, Rognum TO, Giercksky KE, Skotheim RI, Lothe RA. **Gene expression profiles of primary colorectal carcinomas, liver metastases and carcinomatoses**. Mol. Cancer, 6:2, 2007.

BACKGROUND: Despite the fact that metastases are the leading cause of colorectal cancer deaths, little is known about the underlying molecular changes in these advanced disease stages. Few have studied the overall gene expression levels in metastases from colorectal carcinomas, and so far, none has investigated the peritoneal carcinomatoses by use of DNA microarrays. Therefore, the aim of the present study is to investigate and compare the gene expression patterns of primary carcinomas (n = 18), liver metastases (n = 4), and carcinomatoses (n = 4), relative to normal samples from the large bowel. RESULTS: Transcriptome profiles of colorectal cancer metastases independent of tumor site, as well as separate profiles associated with primary carcinomas, liver metastases, or peritoneal carcinomatoses, were assessed by use of Bayesian statistics. Gains of chromosome arm 5p are common in peritoneal carcinomatoses and several candidate genes (including PTGER4, SKP2, and ZNF622) mapping to this region were overexpressed in the tumors. Expression signatures stratified on TP53 mutation status were identified across all tumors regardless of stage. Furthermore, the gene expression levels for the in vivo tumors were compared with an in vitro model consisting of cell lines representing all three tumor stages established from one patient. CONCLUSION: By statistical analysis of gene expression data from primary colorectal carcinomas, liver metastases, and carcinomatoses, we are able to identify genetic patterns associated with the different stages of tumorigenesis.

Leithe E and Rivedal E. **Ubiquitination of gap junction proteins**. J. Membr. Biol., 217: 43-51, 2007.

Gap junctions are plasma membrane domains containing arrays of channels that exchange ions and small molecules between neighboring cells. Gap junctional intercellular communication enables cells to directly cooperate both electrically and metabolically. Several lines of evidence indicate that gap junctions are important in regulating cell growth and differentiation and for maintaining tissue homeostasis. Gap junction channels consist of a family of transmembrane proteins called connexins. Gap junctions are dynamic structures, and connexins have a high turnover rate in most tissues. Connexin43 (Cx43), the best-studied connexin isoform, has a half-life of 1.5-5 h; and its degradation involves both the lysosomal and proteasomal systems. Increasing evidence suggests that ubiquitin is important in the regulation of Cx43 endocytosis. Ubiquitination of Cx43 is thought to occur at the plasma membrane and has been shown to be regulated by protein kinase C and the

mitogen-activated protein kinase pathway. Cx43 binds to the E3 ubiquitin ligase Nedd4, in a process modulated by Cx43 phosphorylation. The interaction between Nedd4 and Cx43 is mediated by the WW domains of Nedd4 and involves a proline-rich sequence conforming to a PY (XPPXY) consensus motif in the C terminus of Cx43. In addition to the PY motif, an overlapping tyrosine-based sorting signal conforming to the consensus of an YXXphi motif is involved in Cx43 endocytosis, indicating that endocytosis of gap junctions involves both ubiquitin-dependent and -independent pathways. Here, we discuss current knowledge on the ubiquitination of connexins.

Lind GE, Skotheim RI, Lothe RA. **The epigenome of testicular germ cell tumors**. APMIS, 115:1147-1160, 2007.

Gene expression is tightly regulated in normal cells, and epigenetic changes disturbing this regulation are a common mechanism in the development of cancer. Testicular germ cell tumor (TGCT) is the most common malignancy among young males and can be classified into two main histological subgroups: seminomas, which are basically devoid of DNA methylation, and nonseminomas, which in general have methylation levels comparable with other tumor tissues, as shown by restriction landmark genome scanning (RLGS). In general, DNA methylation seems to increase with differentiation, and among the nonseminomas, the pluripotent and undifferentiated embryonal carcinomas harbor the lowest levels of DNA promoter hypermethylation, whereas the well-differentiated teratomas display the highest. In this regard, TGCTs resemble the early embryogenesis. So far, only a limited number of tumor suppressor genes have been shown to be inactivated by DNA promoter hypermethylation in more than a minor percentage of TGCTs, including MGMT, SCGB3A1, RASSF1A, HIC1, and PRSS21. In addition, imprinting defects, DNA hypomethylation of testis/cancer associated genes, and the presence of unmethylated XIST are frequent in TGCTs. Aberrant DNA methylation has the potential to improve current diagnostics by noninvasive testing and might also serve as a prognostic marker for treatment response.

Lind GE, Ahlquist T, Lothe RA. **DNA hypermethylation of MAL – a promising diagnostic biomarker for colorectal tumors**. Gastroenterology, 132: 1631-2, 2007.

We found the article by Mori et al[1] recently published in Gastroenterology of great interest. In this paper, several novel promoter methylation target genes were identified in colon cancer and among them, MAL, encoding a T-cell differentiation protein. This gene was also identified as a potential target in a recent study from our group using the same methodologic approach as Mori et al (microarray-based gene expression analyses before and after 5-aza-2'-deoxycytidine treatment of cell lines).[2] Only responding genes with concomitant reduced expression in in vivo tumors were selected for detailed DNA methylation analysis. We report here hypermethylation of MAL in an exceptionally high frequency among malignant (83%; 40 of 48 carcinomas) as well as in benign large bowel tumors (73%; 43 of 59 adenomas) as assessed by methylation-specific polymerase chain reaction (MSP) analysis (Figure 1; primers given on request).

This high methylation frequency of MAL is in contrast to the 6% methylation (2 of 34 samples) reported by Mori et al[1] using real-time MSP. However, they did not perform an independent validation assay for the methylation status of the gene in question, opting for caution concerning the correctness of the initial frequency. By direct bisulphite sequencing of colon cancer cell lines, we have confirmed the DNA methylation status established by MSP in the present report, and show that the majority of CpG sites were indeed methylated in the samples identified as methylated by MSP. Furthermore, the majority of normal mucosa samples were unmethylated, only 2 of 18 normal mucosa samples taken from distant sites from the primary carcinoma, and 1 of 23 normal mucosa samples from large bowels without cancer showed weak methylation (seen as a low-intensity band compared with the positive control after gel electrophoresis). The frequency found among normal samples was significantly less than in primary adenomas (P < .0001) and carcinomas (P < .0001). These results suggest that hypermethylation of MAL is suitable as an early diagnostic marker of primary or recurrent colorectal tumors.

Early detection of disease can result in improved clinical outcome for most types of cancer and identification of cancer-associated aberrant gene methylation represents promising novel biomarkers.[3] For colorectal cancer, initial studies have identified the presence of aberrantly methylated DNA in patient blood and feces. To our knowledge, only 2 of the genes screened for methylation in fecal DNA, VIM (vimentin) and SFRP2, have shown high sensitivity and specificity.[4] and [5] In general, the sensitivity and specificity of existing early markers remain suboptimal, independent of whether they are detecting DNA sequence changes or DNA

modifications by methylation. Genes aberrantly hypermethylated at high frequencies already in benign tumors and only rarely in normal mucosa are good candidate diagnostic biomarkers owing to the potential clinical benefit of early detection of high-risk adenomas as well as early stages of carcinomas. Promoter hypermethylation of MAL, shown to be present in the vast majority of colorectal adenomas and carcinomas and only rarely in normal mucosa, therefore represents another promising early diagnostic marker that should be further studied in fecal and serum DNA samples and possibly included in a panel of biomarkers for noninvasive testing.

Lind GE, Ahlquist T, Kolberg M, Berg M, Eknæs M, Alonso MA, Kallionemi A, Meling GI, Skotheim RI, Rognum TO, Thiis-Evensen E, Lothe RA. **Hypermethylated MAL gene − a silent marker of early colon tumorigenesis**. J. Translational Medicine (BMC "Highly accessed"), 6:13, 2008.

BACKGROUND: Tumor-derived aberrantly methylated DNA might serve as diagnostic biomarkers for cancer, but so far, few such markers have been identified. The aim of the present study was to investigate the potential of the MAL (T-cell differentiation protein) gene as an early epigenetic diagnostic marker for colorectal tumors. METHODS: Using methylation-specific polymerase chain reaction (MSP) the promoter methylation status of MAL was analyzed in 218 samples, including normal mucosa (n = 44), colorectal adenomas (n = 63), carcinomas (n = 65), and various cancer cell lines (n = 46). Direct bisulphite sequencing was performed to confirm the MSP results. MAL gene expression was investigated with real time quantitative analyses before and after epigenetic drug treatment. Immunohistochemical analysis of MAL was done using normal colon mucosa samples (n = 5) and a tissue microarray with 292 colorectal tumors. RESULTS: Bisulphite sequencing revealed that the methylation was unequally distributed within the MAL promoter and by MSP analysis a region close to the transcription start point was shown to be hypermethylated in the majority of colorectal carcinomas (49/61, 80%) as well as in adenomas (45/63, 71%). In contrast, only a minority of the normal mucosa samples displayed hypermethylation (1/23, 4%). The hypermethylation of MAL was significantly associated with reduced or lost gene expression in in vitro models. Furthermore, removal of the methylation re-induced gene expression in colon cancer cell lines. Finally, MAL protein was expressed in epithelial cells of normal colon mucosa, but not in the malignant cells of the same type. CONCLUSION: Promoter hypermethylation of MAL was present in the vast majority of benign and malignant colorectal tumors, and only rarely in normal mucosa, which makes it suitable as a diagnostic marker for early colorectal tumorigenesis.

Oldenburg J, Kraggerud SM, Cvancarova M, Lothe RA, Fosså SD. **Cisplatin-induced long-term hearing impairment is associated with specific glutathione-s-transferase genotypes in testicular cancer survivors**. J. Clin. Oncol., 25: 708-14, 2007.

Purpose: Cisplatin, a cornerstone of combination chemotherapy in the treatment of testicular cancer, induces hearing impairment with considerable interindividual variations. These differences might
be a result of functional polymorphisms in cisplatin-detoxifying enzymes like glutathione
S-transferases (GSTs).
Patients and Methods: We identified 173 cisplatin-treated testicular cancer survivors (TCSs) who had participated in a long-term survey that included audiometric testing and lymphocyte sampling. The hearing decibel thresholds at 4,000 Hz were categorized into leveled scales by normative decibel percentiles. Known functional polymorphisms (positive or negative) in GSTT1 and GSTM1 and codon 105 A/G (Ile/Val) in GSTP1 were analyzed by multiplex polymerase chain reaction, followed by restriction enzyme cutting, and separated by gel electrophoresis.
Results: The risk of having an inferior audiometric result was more than four times higher in TCSs with 105Ile/105Ile-GSTP1 or 105Val/105Ile-GSTP1 compared with 105Val/105Val-GSTP1 (odds ratio [OR] _ 4.21; 95% CI, 1.99 to 8.88; P _ .001 when modeled by ordinal logistic regression [OLR]). GSTM1 positivity was detrimental for hearing ability. Two combined genotypes were associated with hearing ability. The presence of pattern 1 (GSTT1 positive, GSTM1 positive, and 105Ile/105Ile-GSTP1) was associated with hearing impairment (OR _ 2.76; 95% CI, 1.35 to 5.64; P _ .005, OLR). TCSs with pattern 2 (GSTT1 positive, GSTM1 positive, and 105Val/105Val-GSTP1) had better hearing ability than TCSs without this pattern (OR _ 5.35; 95% CI, 2.25 to 12.76; P _ .001, OLR).
Conclusion: The presence of both alleles of 105Val-GSTP1 offered protection against cisplatin-induced hearing

impairment. Two genotype patterns with good and poor protection against cisplatin-induced ototoxicity were identified.

Oldenburg J, Kraggerud SM, Brydøy M, Cvancarova M, Lothe RA, Fosså SD. **Association between long term neurotoxicities in testicular cancer survivors are associated with polymorphisms in glutathione-s-transferase-P1 and-M1, a retrospective cross sectional study**. J. Translational Medicine (BMC), 5:70 (pp), 2007.

BACKGROUND: To assess the impact of polymorphisms in Glutathione S-transferase (GST) -P1, -M1, and -T1 on self-reported chemotherapy-induced long-term toxicities in testicular cancer survivors (TCSs). METHODS: A total of 238 TCSs, who had received cisplatin-based chemotherapy at median twelve years earlier, had participated in a long-term follow-up survey which assessed the prevalence of self-reported paresthesias in fingers/toes, Raynaud-like phenomena in fingers/toes, tinnitus, and hearing impairment. From all TCSs lymphocyte-derived DNA was analyzed for the functional A-->G polymorphism at bp 304 in GSTP1, and deletions in GST-M1 and GST-T1. Evaluation of associations between GST polymorphisms and self-reported toxicities included adjustment for prior treatment. RESULTS: All six evaluated toxicities were significantly associated with the cumulative dose of cisplatin and/or bleomycin. Compared to TCSs with either GSTP1-AG or GSTP1-AA, the 37 TCSs with the genotype GSTP1-GG, were significantly less bothered by paresthesias in fingers and toes (p = 0.039, OR 0.46 [0.22-0.96] and p = 0.023, OR 0.42 [0.20-0.88], respectively), and tinnitus (p = 0.008, OR 0.33 [0.14-0.74]). Furthermore, absence of functional GSTM1 protected against hearing impairment (p = 0.025, OR 1.81 [1.08-3.03]). CONCLUSION: In TCSs long-term self-reported chemotherapy-induced toxicities are associated with functional polymorphisms in GSTP1 and GSTM1. Hypothetically, absence of GST-M1 leaves more glutathione as substrate for the co-expressed GST-P1. Also intracellular inactivation of pro-apoptotic mediators represents a possible explanation of our findings. Genotyping of these GSTs might be a welcomed step towards a more individualized treatment of patients with metastatic testicular cancer.

Ribeiro FR, Skotheim RI, Henrique R, and Teixeira MR. Book chapter 30: **Array-based comparative genomic hybridization in prostate cancer: research and clinical applications**. In: Methods of cancer diagnosis, therapy and prognosis (Hayat MA, Ed.), volume II, Springer Publishers, USA, 2008.

It is widely recognized that acquired genomic aberrations, leading to loss of function of tumor suppressor genes or gain of function of protooncogenes, are the driving force behind cellular neoplastic transformation (Hanahan and Weinberg, 2000). The increased knowledge of the human genome and the advances in the field of biotechnology have provided us with powerful tools for high-throughput characterization of these alterations in cancer samples. Novel microarray platforms with genome-wide coverage at escalating resolutions give promise of quickly uncovering the genetic events driving neoplastic transformation, which have previously gone undetected.
In this chapter we summarize the stateof- the-art in the fast-evolving field of genomic microarrays, highlighting some of the advantages and pitfalls of different array platforms and analyses techniques. We also review the relative contribution of this new technology to the knowledge of prostate cancer genetics, with an emphasis on its potential use in a clinical setting.

Rivedal E, Leithe E. **The benzene metabolite trans,trans-muconaldehyde blocks gap junction intercellular communication by cross-linking connexin43**. Toxicol. Appl. Pharmacol., 232:463-8, 2008.

Benzene is used at large volumes in many different human activities. Hematotoxicity and cancer-causation as a result of benzene exposure was recognized many years ago, but the mechanisms involved remain unclear. Aberrant regulation of gap junction intercellular communication (GJIC) has been linked to both cancer induction and interference with normal hematopoietic development. We have previously suggested that inhibition of GJIC may play a role in benzene toxicity since benzene metabolites were found to block GJIC, the ring-opened trans,trans-muconaldehyde (MUC) being the most potent metabolite. In the present work we have studied the molecular mechanisms underlying the MUC-induced inhibition of gap junctional communication. We show that MUC induces cross-linking of the gap junction protein connexin43 and that this is likely to be responsible for the induced inhibition of GJIC, as well as the loss of connexin43 observed in Western blots. We also show that glutaraldehyde possesses similar effects as MUC, and we compare the effects to that of formaldehyde. The fact that glutaraldehyde and formaldehyde have been associated with induction of

leukemia as well as disturbance of hematopoiesis, strengthens the possible link between the effect of MUC on gap junctions, and the toxic effects of benzene.

Røyrvik E, Ahlquist T, Rognes T, Lothe RA. **„Slip Slidin' Away: A duodecennial review of targeted genes in mismatch repair deficient colorectal cancer"** invited review, CRO-Critical Reviews in Oncogenesis, 13: 229-257, 2007. Front cover.

Roughly 15% of colorectal tumors are characterized by microsatellite instability (MSI), a deficiency caused by defective DNA mismatch repair, which leads to profuse insertions and deletions in microsatellites. Downstream target genes of this defective repair are those prone to exhibit these insertion/deletion mutations in their coding regions and potentially having functional consequences in, and providing a growth advantage for, the cancer cell. This review presents the last 12 years of research on these MSI target genes, systematizing the mutation details of the more than 160 genes identified to date, and includes their mutation frequencies in colorectal and other MSI (e.g., gastric and endometrial) tumors. Functional aspects of certain targets and the target gene concept itself are also discussed, as is the comparative wealth of potential target genes assessed by scanning the coding sequences of the human genome for mononucleotide repeats--yet to be investigated.

Sirnes S, Leithe E, Rivedal E. **The detergent resistance of Connexin43 is lost upon TPA or EGF treatment and is an early step in gap junction endocytosis**. Biochem. Biophys. Res. Commun., 373:597-601, 2008.

Gap junctions are plasma membrane domains containing channels that directly connect the cytosols of neighbouring cells. Gap junction channels are made of a family of transmembrane proteins called connexins, of which the best studied is Connexin43 (Cx43). MAP kinase-induced phosphorylation of Cx43 has previously been shown to cause inhibition of gap junction channel permeability and increased Cx43 endocytosis. As Cx43 assembles into gap junction plaques, Cx43 acquires detergent resistance. Here we report that the detergent resistance is lost after activation of MAP kinase. Treatment of IAR20 rat liver epithelial cells with 12-O-tetradecanoylphorbol 13-acetate (TPA) or epidermal growth factor (EGF) caused a rapid increase in the solubility of Cx43 in Triton X-100. This process was mediated by MAP kinase and was initiated at the plasma membrane. The data suggest that loss of the detergent resistance of Cx43 is an early step in TPA- and EGF-induced endocytosis of gap junctions.

Skotheim RI and Nees M. **Alternative splicing in cancer: noise, functional, or systematic?** Int. J. Biochem. Cell Biol, 39: 1432-49, 2007.

Pre-messenger RNA splicing is a fine-tuned process that generates multiple functional variants from individual genes. Various cell types and developmental stages regulate alternative splicing patterns differently in their generation of specific gene functions. In cancers, splicing is significantly altered, and understanding the underlying mechanisms and patterns in cancer will shed new light onto cancer biology. Cancer-specific transcript variants are promising biomarkers and targets for diagnostic, prognostic, and treatment purposes. In this review, we explore how alternative splicing cannot simply be considered as noise or an innocent bystander, but is actively regulated or deregulated in cancers. A special focus will be on aspects of cell biology and biochemistry of alternative splicing in cancer cells, addressing differences in splicing mechanisms between normal and malignant cells. The systems biology of splicing is only now applied to the field of cancer research. We explore functional annotations for some of the most intensely spliced gene classes, and provide a literature mining and clustering that reflects the most intensely investigated genes. A few well-established cancer-specific splice events, such as the CD44 antigen, are used to illustrate the potential behind the exploration of the mechanisms of their regulation. Accordingly, we describe the functional connection between the regulatory machinery (i.e., the spliceosome and its accessory proteins) and their global impact on qualitative transcript variation that are only now emerging from the use of genomic technologies such as microarrays. These studies are expected to open an entirely new level of genetic information that is currently still poorly understood.

Wu Q, Lothe RA, Ahlquist T, Silins I, Tropé CG, Micci F, Nesland JM, Suo Z, Lind GE. **DNA methylation profiling of ovarian carcinomas and their in vitro models identifies HOXA9, HOXB5, SCGB3A1, and CRABP1 as novel targets**. Mol. Cancer, 6:45 (pp.10), 2007 (BMC: Flagged as "Highly accessed")

BACKGROUND: The epigenetics of ovarian carcinogenesis remains poorly described. We have in the present study investigated the promoter methylation status of 13 genes in primary ovarian carcinomas (n = 52) and their in vitro models (n = 4; ES-2, OV-90, OVCAR-3, and SKOV-3) by methylation-specific polymerase chain reaction (MSP). Direct bisulphite sequencing analysis was used to confirm the methylation status of individual genes. The MSP results were compared with clinico- pathological features. RESULTS: Eight out of the 13 genes were hypermethylated among the ovarian carcinomas, and altogether 40 of 52 tumours were methylated in one or more genes. Promoter hypermethylation of HOXA9, RASSF1A, APC, CDH13, HOXB5, SCGB3A1 (HIN-1), CRABP1, and MLH1 was found in 51% (26/51), 49% (23/47), 24% (12/51), 20% (10/51), 12% (6/52), 10% (5/52), 4% (2/48), and 2% (1/51) of the carcinomas, respectively, whereas ADAMTS1, MGMT, NR3C1, p14ARF, and p16INK4a were unmethylated in all samples. The methylation frequencies of HOXA9 and SCGB3A1 were higher among relatively early-stage carcinomas (FIGO I-II) than among carcinomas of later stages (FIGO III-IV; P = 0.002, P = 0.020, respectively). The majority of the early-stage carcinomas were of the endometrioid histotype. Additionally, HOXA9 hypermethylation was more common in tumours from patients older than 60 years of age (15/21) than among those of younger age (11/30; P = 0.023). Finally, there was a significant difference in HOXA9 methylation frequency among the histological types (P = 0.007). CONCLUSION: DNA hypermethylation of tumour suppressor genes seems to play an important role in ovarian carcinogenesis and HOXA9, HOXB5, SCGB3A1, and CRABP1 are identified as novel hypermethylated target genes in this tumour type.

Wu Q, Lind GE, Aasheim HC, Silins I, Tropé CG, Nesland JM, Lothe RA, Suo Z. **The EPH receptor Bs (EPHBs) promoters are unmethylated in colon and ovarian cancers**. Epigenetics, 2 (4): 237-243, 2007.

Aberrant expression of EPH receptors and their ligands, ephrins, has been reported in a large variety of human cancers, including epithelial cancers from the colon and ovary. Due to the recently reported decrease or loss of EPHBs expression in colorectal carcinomas and the abundance of CpG sites in their promoters, we analyzed the promoter methylation status of three members of the EPHB family, EPHB2, EPHB3 and EPHB4, in a series of 22 colon cancer cell lines, as well as in four ovarian cancer cell lines and 56 ovarian tumor samples. The promoters of the three receptor genes were unmethylated in the vast majority of samples as assessed by methylation-specific polymerase chain reaction (MSP). These results were confirmed by direct bisulphite sequencing. Furthermore, from RT-PCR analyzes and Northern blotting, EPHB2 showed only small variation in RNA expression across ovarian cancer cell lines and clinical samples. We conclude that promoter hypermethylation of EPHB2, EPHB3 and EPHB4 is not a common event in colon and ovarian cancers and therefore plays no major role in these tumors.