Acta Oncologica Vol. 37, No. 3, pp. 307–314

Abstracts of Theses from the Scandinavian Countries

Abstracts of Scandinavian theses on oncologic subjects are published under this heading. The full theses are as a rule published by the universities or as supplements to different journals. They can usually be obtained after contact with the author.

Characterization of the perturbed differentiation in neuroblastoma, aided by the analysis of normal sympathetic nervous system development

Carolina Gestblom

Division of Molecular Medicine, Department of Laboratory Medicine, Universitetssjukhuset MAS, Lund University, S-205 02 Malmö, Sweden

Neuroblastoma is a pediatric tumor derived from cells in the sympathetic nervous system. In an attempt to explain the heterogeneous clinical behavior of neuroblastoma tumors, neuroblastomas have been characterized with regard to their degree of sympathetic differentiation. Their expression of genes and proteins that regulate normal sympathetic development and cell cycle/cell death regulators was assessed by *in situ* hybridization and immunohistochemistry. The cell types of the developing prenatal sympathetic nervous system were also characterized in order to identify marker genes that distinguish between neuronal and neuroendocrine symathetic cell types. Principally two groups of neuroblastomas have been identified. Unfavorable prognosis, nondifferentiating neuroblastomas expressed marker genes of immature sympathetic neuroblasts. Differentiating neuroblastoma tumors, often with favorable prognosis, also expressed neuronal marker genes, but only in the immature, proliferating cells adjacent to the fibrovascular stroma. With increasing distance from the stroma, these tumor cells cease to proliferate, downregulate their expression of neuronal marker genes, differentiate, and upregulate their expression of neuroendocrine marker genes. Thus, it appears that in these tumors initially neuroblastic cells mature towards a neuroendocrine lineage. Also cell cycle and cell death regulators were expressed in a physiological context resembling normal cellular behavior in these tumors. Proliferating cells expressed Bcl-2. With differentiation, cells downregulate Bcl-2 and upregulate p53. Cells adjacent to the p53 expressing cells die by apoptosis. Karyorrhectic cells, as defined by the mitosis-karyorrhexis index, were identified to be either proliferating or apoptotic. Neuroblastoma cell lines, established from high stage tumors, were analyzed with regard to their unability to respond to nerve growth factor (NGF). These cell lines expressed the high-affinity NGF receptor TrkA and the low-affinity receptor, and yet they did not respond to NGF. By the introduction of exogenous TrKA, NGF-responsiveness was partly restored in these cell lines.

April 1997

Prognostic factors in prostate cancer

Pär Stattin

Departments of Urology & Andrology, and Pathology, Umeå University, S-901 85 Umeå, Sweden

The clinical course of prostate cancer (PCa), the most common cancer in Swedish men, is highly variable and difficult to predict. Consequently, there is an urgent need to distinguish tumours with a high risk of progression from those with a low risk. To investigate the prognostic implications of proliferation and apoptosis, two important processes in tumour biology, immunoreactivity for biomarkers associated with these processes was assessed, quantified in indexes, and related to cause-specific survival (CSS).

A consecutive series of 186 men presenting with voiding symptoms and PCa were treated with transurethral resection and deferred endocrine therapy. After 13–21 years follow-up, 43% of these men had died of prostate cancer. In a subgroup of men with localised disease at the time of diagnosis, 27% succumbed to the disease. Immunoreactivity for p53 protein, indicative of a defective p53 function, predicted shorter CSS in univariate (52 vs 123 months, $p < 0.0001$), but not in multivariate analysis.

Mean index for the apoptosis blocking bcl-2 protein was higher in foci of prostatic intraepithelial neoplasia, a putative precursor to PCa, than in manifest cancer areas (79 vs 12, $p < 0.0001$). This indicates that bcl-2 may be involved in early tumourigenesis. No prognostic value was found for the bcl-2 index. A high index for the proliferation marker Ki-67 predicted shorter CSS in univariate (53 vs 132 months $p < 0.0001$) and in multivariate analysis. To test if p53 is predictive for clinical radioresistance, as suggested by experimental models, p53 immunoreactivity was investigated in biopsies obtained before radical radiotherapy in an unrelated series of 60 PCa patients. Patients with p53 reactive tumours had longer CSS, indicating that p53 is not treatment-predictive for radiotherapy in Pca.

Core biopsies were obtained before and a week after castration therapy in patients with advanced PCa. According to the serum prostate specific antigen (PSA) level 3 months after therapy, 15 responding tumours and 13 non-responding tumours were selected. Regressive morphology was seen in 14/15 responders after castration therapy, compared with 4/13 non-responders. Median apoptotic index increased significantly after castration therapy for responders (from 2.6 to 3.5, $p < 0.05$) whereas it was 2.8 before and after therapy in non-responders. This indicates that subsequent clinical response can be predicted by the induction of regressive morphology and an increase in apoptotic index.

In conclusion, immunoreactivity for Ki-67 appeared to be a putative prognostic factor in PCa, whereas the prognostic value of p53 and bcl-2 was dubious. p53 immunoreactivity did not appear to be predictive of radioresistance in PCa. Cellular response in biopsies shortly after castration therapy might be treatment-predictive.

May 1997

Estrogen receptor gene alterations in human breast cancer

Qui-Xia Zhang

Jubileumsinstitutionen, Department of Oncology, University Hospital, S-221 85 Lund, Sweden

Estrogens play important roles in the growth and development of both normal and malignant breast tissue. The presence of estrogen receptors (ER) in breast cancer is an indicator of hormone-sensitivity and a predictor of hormone therapy efficacy. However, a significant part of ER + breast tumors fails to respond to tamoxifen therapy, and many initially responsive tumors eventually acquire an endocrine resistance. It has been suggested that variants and mutations in the ER gene may be involved in this process. The ER locus on chromosome 6q25.1 has also been implicated in an inherited predisposition for breast cancer, although evidence of the specific ER gene lesions involved has been lacking. Germline mutations in the androgen receptor (AR) gene have though been identified in males with breast cancer.

Thus, the aims of the present thesis were to study the involvement of both the ER and AR genes in breast cancer development, progression and resistance to endocrine therapy, as well as their importance in hereditary breast cancer. Different splicing variants of the ER transcript were found to be abundant in breast cancer cell lines and breast tumors and their presence was not related to efficacy of adjuvant endocrine treatment. ER gene mutations were found to be rare in primary breast tumors, but the presence in a metastatic breast tumor of an ER mutant with strong constitutive transcriptional activity suggests a role of ER alterations in tumor progression. The presence of ER variants in premalignant lesions at different stages of breast tumor evolution has been analysed, and one ER variant manifesting increased sensitivity to estrogen stimulation may be important in the development of breast hyperplasias. A germline ER mutation was found in a male with breast cancer and a family history of the disease, suggesting that the ER gene should be included in the increasingly larger family of breast cancer susceptibility genes.

May 1997

Boron neutron capture therapy—Biological evaluation of new compounds and conjugates

PÄR OLSSON

Biomedical Radiation Sciences, Uppsala University, Box 535, S-751 21 Uppsala, Sweden

Boron neutron capture therapy (BNCT) is based on the selective association of ^{10}B with cancer cells. Upon capture of thermal neutrons ^{10}B, high energy He and Li particles are emitted. If a sufficiently high local concentration of ^{10}B can be achieved, cells loaded with ^{10}B will be killed, whereas cells outside the field of neutron irradiation will remain intact. ^{10}B can be delivered to tumour cells either directly, with tumour-specific boron compounds, or via the coupling of boron compounds to tumour targeting agents. In this work, some biological properties of eight new carborane-based boron compounds and two epidermal growth factor (EGF)—dextran conjugates were investigated.

The diol amino carborane, DAC-1, showed high accumulation in cells. The concentration of boron in the cells was up to 90 times higher than in the culture medium. DAC-1 exposed cells also showed a long cellular retention of boron. About 50% of the initial cell-associated boron was still retained in the cells after 4 days. Neither DAC-1 nor an amino-acid carborane named DAAC-1, showed uptake in cell nuclei. In *in vitro* therapy studies it was found that, after neutron activation, DAC-1 had better cell-inactivating capability than both DAAC-1 and the clinically used compound boronophenylalanine, BPA.

The low molecular weight boron compound sulfhydryl boron hydride, BSH, was coupled to EGF-dextran for targeting against tumour cells with an overexpression of EGF-receptor. Up to 800 boron atoms were coupled to each conjugate. Theoretically, this means that the requirements for a significant BNCT effect is within reach.

The two analysed conjugates showed specific binding to EGF-receptors *in vitro*, and the dextran part of the conjugates seemed to stabilize the EGF part.

The EGF-dextran-BSH conjugate showed receptor-dependent binding to EGF-receptor rich cells *in vivo*. It was also found that problems with high liver, and low tumour, uptake could be circumvented to some degree by changing the administration of the conjugate, from intravenous to intratumoral injection.

May 1997

Interstitial laser thermotherapy of liver tumours. Methodology and application

PÅLL HELGI MÖLLER

Department of Surgery, Lund University Hospital, S-221 85 Lund, Sweden

Interstitial laser thermotherapy (ILT) is a method well suited for selective local destruction of malignant tumours. In the present work, a laser thermotherapy system based on feedback temperature control of energy delivery (through computerised feedback system) was developed. Temperature control was excellent in experiments without carbonization and with preserved light penetration, whereas temperature oscillations were large in experiments with carbonization and impaired light penetration. Using bare fibre, carbonization was seen in almost all experiments *in vitro*, regardless of output power (1–4 W) but only occasionally *in vivo* using 2 W. Treatment with a sapphire probe, representing a diffuser tip, gave carbonization at higher output powers (>3 W *in vitro*, >4 W *in vivo*). The bare fibre gave a steeper temperature gradient than the sapphire probe. In normal pig liver the volume of thermal damage was increased five times by occluding the blood flow to the liver during treatment. It was not possible to predict the size of thermal damage in normal pig livers using ultrasonography. The effect of different temperatures and different treatment times was studied in rats with transplanted liver tumours. It appeared that total tumour necrosis was obtained with a temperature of 54–61°C at the tumour margin and a treatment time of 30 min. In a subsequent study, ILT (46°C for 30 min) was based on these findings and compared with resection of the tumour-bearing liver lobe. There was no difference in local tumour control but the incidence and extent of intraabdominal spread was lower after ILT than after resection. ILT was used in patients with irresectable tumours, and problems concerned with monitoring of thermal damage and limited volume effect were identified.

May 1997

The fluorometric microculture cytotoxicity assay (FMCA) in assessment of the clinical activity of cytotoxic drugs—With special reference to ovarian carinoma

KATALIN CSÓKA

Division of Clinical Pharmacology, University Hospital, S-751 85 Uppsala, Sweden

Selection of cytotoxic drugs for individual patients with malignant tumors is mostly based on results from large clinical trials. There is reason to believe that cancer chemotherapy and new drug development could be optimised by the use of valid *in vitro* tests for tumor cell drug sensitivity. The present investigation was undertaken to evaluate the potential role of the Fluorometric Microculture Cytotoxicity Assay (FMCA) for these purposes and focused on ovarian carcinoma and other solid tumors.

By the combined use of mechanical and enzymatic digestion followed by purification on Ficoll and/or Percoll density gradients, tumor representative cell preparations suitable for the FMCA were obtained from a majority of patient samples and tumor types. The versatile technique of tumor sampling by ultrasound guided biopsy seemed to be feasible for obtaining tumor cells for analysis in the FMCA.

Results from the FMCA correlated well with the established DiSC assay and the fluorescence from non-malignant cells was

low compared with that of tumor cells. Ovarian carcinoma was found to be feasible in the FMCA and the sensitivity pattern reported for standard drugs in this and other tumor diagnoses was in good agreement with clinical experience.

The newly introduced antimetabolite gemcitabine showed *in vitro* activity close to that of ara-C, with pronounced activity in hematologic tumors. However, gemcitabine showed slight activity also in solid tumors, as suggested from the clinic. The old antiparasitic agent, suramin, showed activity in clinically highly resistant solid tumors. The activity pattern of Taxol *in vitro* was compatible with that found in the clinic. However, most of its effect *in vitro* was mediated by its excipient, cremophor EL.

In 45 patients with ovarian carcinoma, clinical outcome was correlated with drug sensitivity reported in the FMCA. Although the assay performance was found inferior to that reported for hematologic tumors, the assay identified the majority of patients as responders and non-responders in the clinic.

The results suggest that the FMCA is feasible also for testing tumor cells from solid tumors. The assay may become a useful tool for optimisation of chemotherapy as well as for evaluation of new drugs.

May 1997

Nonradioactive quantitative detection of gene expression—Methodological studies in normal and malignant tissues utilizing digital image analysis

Thomas Averell Houze

Department of Surgery, University of Gothenburg, Sahlgren's University Hospital/Östra, Oncology Laboratory-2, S-416 85 Gothenborg, Sweden

This thesis describes the development and adaptation of gene expression analysis to archival biopsies, allowing retrospective investigation of thymidylate synthase (TS) gene expression without the need for preemptive sample recovery. The ability to effectively isolate messenger ribonucleic acid (mRNA) from formalin-fixed paraffin embedded material for RNA analysis, and the nearly universal accessibility of this material opens the archives of hospital pathology departments to all sorts of gene expression studies. The methods developed in this thesis are based on the reverse-transcriptase polymerase chain reaction (RT-PCR) amplification of complimentary deoxyribonucleic acid (cDNA) obtained from different tissues. The quantification of the PCR amplicons is by computer assisted digital image analysis (DIA). As model systems five different processes are investigated including the development of a practical DIA workstation for nonradioactive detection of gene expression. The workstation is first applied to the analysis of the transcriptional regulation of interferon-gamma (IFN-γ) gene expression in interleuken-2 (IL-2) stimulated natural killer cells (NK) by histamine and catalase in the presence and absence of autologous monocytes. The same system is then applied to the analysis of TS gene expression in flash-frozen biopsies. Next, a solid-phase system of isolating and concentrating mRNA from formalin-fixed paraffin embedded biopsies with reusable oligo$(dT)_{25}$ paramagnetic beads was developed. The method uses the repeated re-elution and concentration of mRNA isolated from the same portion of an archival biopsy.

Intracellular concentrations of TS protein are studied in surgical biopsies from normal and cancerous human tissue originating from the GI-tract prior to the administration of 5FU and compared to TS gene expression. The concentration of TS is measured with tritium release assays, and the TS gene expression levels for both flash-frozen and formalin-fixed archival tissues are determined with the PCR based methodology previously described. The concentrations of TS mRNA varied considerably between the different tumor types examined, however the TS concentrations were consistently higher in tumor than in normal tissue. The use of oligo$(dT)_{25}$ paramagnetic beads allowed for the isolation of intact mRNA even from archival tissues high in ribonucleic acidase (Rnase) activity such as liver. The TS gene expression levels strongly correlated to enzyme activity for both flash-frozen and formalin-fixed material.

In conclusion, these new methodologies should allow for the possibility to accurately determine TS gene expression using archival material in large retrospective studies. The use of competitive semi-quantitative RT-PCR, the 96-well microtiter format and multi-channel pipette with a 'structured' pipetting protocol throughout each experiment were permissible in a clinical setting and proved not only amenable to the potential large-scale screening of biopsy material but permitted greater precision, accuracy and reproducibility by reducing the number of steps required to perform an experiment.

May 1997

Treatment of localised prostatic carcinoma with reference to radiotherapy treatment methods, local cure, late side effects and development of a disease specific quality of life questionnaire

Göran Borghede

Department of Oncology, Sahlgrenska University Hospital, Göteborg, Sweden

Aims: To investigate the ability of external beam radiotherapy (EBRT) to achieve local cure in localised prostate cancer as proven by biopsy and serum PSA. To describe the treatment-related toxicity as estimated by the physicians and the patients. To develop an improved radiotherapy technique suitable for localised prostate cancer. To develop a prostate cancer specific module supplementing a core quality of life questionnaire and covering essential aspects of the disease and its treatments.

Methods: Consecutive non-metastatic patients were irradiated with EBRT, 64–70 Gy, or with a combination of external beam irradiation (50 Gy) and brachytherapy (BT) (20 Gy to the prostate) and followed prospectively with respect to local outcome evaluation and toxicity (modified RTOG scale). Cox regression analysis was used for analysis of factors contributing to local control and toxicity outcome. The quality of life investigations were performed as mailout-mailback cross-sectional studies in a sample of irradiated patients and further in an unselected prostate cancer population. Psychometric analyses comprised item convergent and discriminant validity, internal consistency reliability of multi-item scales and the validity of the scales for correlation analysis of clinical relevance.

Results: No evidence of cancer was detected by digital rectal examination in 171 (98%) of 175 patients after EBRT. Transrectal ultrasound (TRUS) guided biopsies performed approximately 18 months after treatment were free of cancer in 75% and a serum prostate-specific antigen (PSA) ≤ 1.0 ng/ml was seen in 66% of the patients. Tumour size and a sufficient treatment margin towards the rectum were established as the most important factors for local control. After EBRT of 184 patients and a follow-up period of 46 months late side effects graded as mild were seen in 53% (gastrointestinal 42%, urogenital 23%) and as moderate to severe in 9% of the patients. A persisting improvement of mild toxicity was experienced by nearly half of the patients. Existing pretreatment symptoms from organs at risk increased the proba-

bility of late toxicity. The combined treatment (EBRT + BT) could be given with good precision and with a biopsy verified local control in 48/50 (96%) and a nadir serum-PSA $\leq$ 1.0 ng/ml in 84% of the patients. Late toxicity was minimal. The quality of life questionnaire was well accepted (response rate > 80%) and the quality of life scores among prostate cancer patients were higher than in patients with other malignancies. The new prostate cancer-specific sexuality and urinary symptom scales were psychometrically robust and clinically relevant. The bowel symptom scale, however, was less specific indicating the neeed for scaling refinement.

Conclusion: According to these non-randomised studies with an intermediate follow-up period, the local cure rate for patients irradiated with BT in combination with EBRT were favourable compared to patients treated with EBRT only. The toxicity risk of both treatments was rather low. The standardised questionnaire techniques in unselected prostate cancer patients provide a normative database for clinical comparisons.

May 1997

Physical mapping and cloning of the t(3;8) (p21;q12) translocation breakpoints in pleomorphic adenomas

Eva Röijer

Laboratory of Cancer Genetics, Department of Pathology, Göteborg University, Sahlgrenska University Hospital, S-413 45 Göteborg, Sweden

The pleomorphic adenoma is a benign epithelial tumor originating from the major and minor salivary glands. It is the most common type of salivary gland tumor, accounting for almost half of all neoplasms in these organs. Cytogenetically, pleomorphic adenomas are characterized by recurrent rearrangements involving the chromosome regions 3p21, 8q12 and 12q13–15. The most frequent abnormality is a reciprocal t(3;8) (p21;q12) translocation, or variants thereof in which the 8q segment is translocated to a variety of chromosome regions. The purpose of the present series of investigations was to identify the genes involved in the t(3;8) (p21;q12) using a positional cloning approach.

YAC clones corresponding to known genetic markers flanking band 8q12 were used to initiate a walk towards the 8q12 breakpoints. This resulted in the establishment of two non-overlapping YAC contigs covering approximately 75% of band 8q12. The centromeric contig covers approximately 2 Mb of genomic DNA and consists of 34 overlapping YAC clones containing at least seven putative CpG islands, and three ESTs. The telomeric contig consists of 23 YACs and covers about 5 Mb of genomic DNA. FISH mapping of YACs and cosmids from these contigs revealed that the majority of breakpoints clustered within a 300 kb subregion in the centromeric contig. Subsequent studies of new STSs and ESTs from this region led to the discovery of the *PLAG1* gene, a novel, developmentally regulated gene coding for a zinc finger protein.

The gene, which consists of 5 exons of which the first three are non-coding, spans about 35 kb, with a large intron (approximately 25 kb) between exon 1 and exon 2. The deduced amino acid sequence of the PLAG1 protein reveals seven canonical C_2H_2 zinc finger domains in the N-terminal region and a serine-rich C-terminus. There are two potential nuclear localization signals in the N-terminal region. 5′ RACE analysis of tumors with t(3;8) enabled us to identify *CTNNB1* as the chromosome 3 gene fused to *PLAG1*. *CTNNB1* codes for β-catenin, a protein interface functioning in the WG/WNT signalling pathway and in the specification of cell fate during embryogenesis.

The t(3;8) results in promoter swapping between *PLAG1* and *CTNNB1*. Fusions invariably occur in the 5′ non-coding regions of both genes, exchanging regulatory control elements while preserving the coding sequences. Due to the t(3;8) *PLAG1* is activated whereas the expression of *CTNNB1* is reduced. Activation of *PLAG1* was also observed in an adenoma with a t(8;15) (q12;q14) translocation, demonstrating that *PLAG1* activation is not restricted to tumors with t(3;8). To our knowledge this is the first example of promoter swapping in solid tumors.

The identification and cloning of a novel 'benign oncogene' activated by chromosome translocation in pleomorphic adenomas constitute an important step towards an increased understanding of the molecular pathogenesis of benign neoplastic growth. The results potentially allow for the design of novel diagnostic tools and targeted therapy of pleomorphic adenomas.

May 1997

Prostate cancer treatment and quality of life—A three-level epidemiological approach

Ásgeir R. Helgason

Radiumhemmet, Karolinska Hospital, Stockholm, Sweden

The decision whether or not to treat localized prostate cancer with curative intent is influenced by the expected beneficial and harmful effects in terms of patient survival and quality of life. In this thesis symptoms specifically related to prostate cancer treatment are investigated, including urinary and bowel symptoms and waning sexual functions. The prevalence of these *symptoms* and the extent to which they distress the men were assessed. Finally, the relationship between *distress* owing to these symptoms and psychological, physical and overall *well-being* was analyzed. Applying epidemiological methods, a total of 842 men were analyzed in seven studies. In four of the studies, 342 prostate cancer patients were compared to a reference group of 314 age matched men without prostate cancer and patients subjected to different treatment protocols were compared to each other and to patients not subjected to any initial treatment.

Urinary and bowel symptoms were more prevalent in the prostate cancer group than in the age-matched reference group but few men, with or without prostate cancer, reported severe symptoms. A small minority of patients (4%) reported high levels of distress owing to urinary or bowel symptoms and their feeling of well-being was impaired. The prevalence of *impaired sexual function* was much higher in the prostate cancer group than in the reference group. The majority of men with the disease that had experienced waning sexual function stated that this distressed them and distress owing to waning sexual function was more common than distress owing to urinary and bowel symptoms. Prostate cancer patients reporting moderate or high distress owing to waning sexual function (52%) reported an impaired feeling of well-being compared with other patients. Men treated for their prostate cancer were more likely to report waning sexual function compared with patients reporting no treatment. External beam radiation therapy was less likely than radical prostatectomy to be related to waning sexual function after treatment. The willingness to *trade off* an intact sexual capacity for the possibility of a prolonged life expectancy varied considerably among the men. This emphasizes the need to adapt treatment decisions to each individual, not only based on medical variables but also on the patient's emotional values and religious and ethical positions. It is possible that the question 'How should localized prostate cancer be treated?' may never be answered. We can only collect valid information about the basis for the trade-off and learn to communicate the data to the patient in a fruitful way.

May 1997

Molecular genetics of hereditary non-polyposis colorectal cancer

PIA TANNERGÅRD

Department of Molecular Medicine, Karolinska Institute, Stockholm, Sweden

Colorectal cancer (CRC) is one of the most prevalent malignancies in the Western World and one of the most predominant causes of death by cancer. There is a subgroup of syndromes with a high incidence of CRC which is transmitted in an autosomal dominant fashion. The most common of these syndromes is hereditary non-polyposis colorectal cancer (HNPCC) which has an estimated incidence of 5%. Families with HNPCC are also at risk of developing extracolonic tumors of the endometrium, stomach, ovary, small intestine, kidney and ureter.

To identify genes predisposing to HNPCC, linkage studies were performed using RFLP marker from all chromosomes and microsatellite markers chromosome 3p. Linkage was found in one Swedish family with the marker D3S1029 on chromosome 3p (paper I). The calculated lod score for the particular family was 3.01. We also observed that tumor DNA tested with microsatellite markers frequently showed gain of extra alleles, not present in the constitutional DNA. The instability phenomenon, later called RER, for replication error, gave a clue to the function of the localized gene (paper I).

The candidate locus on the short arm of chromosome 3 was further sublocalized using 19 microsatellite markers. The markers were mapped in relation to each other by the use of rodent cell hybrids containing the human chromosome 3 with various deletions. Using two families linked to the 3p locus for haplotype analysis, the putative disease gene location was mapped to 3p21.3-23 (paper II).

The *hMLH1* gene, involved in DNA mismatch repair, was located to chromosome 3p and identified to cause HNPCC. Denaturing gradient gel electrophoresis (DGGE) was used to screen 39 Swedish HNPCC families for disease causing mutations in the *hMLH1* gene. Eight germline mutations and 3 polymorphisms were identified. Two of the mutations were splice mutations resulting in a deletion of an exon in the HMLH1 transcript and 6 were missense mutations, changing one amino acid for another in the protein (paper III). The same set of 39 families, as well as 22 new families, were tested for the most common Finnish founder mutation. Three families, all of Finnish origin, were shown to have this genomic deletion of *hMLH1* (paper IV).

Colorectal tumors from patients with HNPCC were shown to have the same frequency of *APC* and *K-RAS* mutations as sporadic CRC. RER was shown to be very frequent, 96% and TGFβ type II receptor (*TβRII*) mutations occur in 56% of the CRC. Tumors from patients with known *hMLH1* germline mutations had lost one allele, indicating that inactivation of both alleles is necessary for defective mismatch repair (paper V). Extracolonic cancers, breast and other tumors, from HNPCC also display a high frequency (69%) of RER, suggesting that these cancers belong to the HNPCC syndrome. One woman with breast cancer, has a constitutional missense mutation in exon 2 of the hMHL1 gene and also a missense mutation in exon 12, which was inherited from her father who also developed breast cancer. To further study this genotype-phenotype correlation we screened 218 breast cancer families for mutations in exon 12 of *hMLH1*. However none of the families presented such a mutation (paper VI).

May 1997

Genomic imprinting and DNA epigenetic modification in human liver and carcinogenesis: regulation of the *IGF2* and *H19* genes

B.M. XURI LI

Department of Clinical Neuroscience, Experimental Alcohol and Drug Addiction Research Section, Laboratory of Molecular Development and Tumor Biology, Karolinska Institute, Stockholm, Sweden

Genomic imprinting, which is a non-classical genetic event discovered approximately a decade ago, has been shown to be involved in the mammalian development and several kinds of human diseases. Epigenetic modifications, such as DNA methylation, are believed to be markers functioning as the imprinting signals. With more and more studies focused in this field, imprinting alteration has been found to be involved in some of the genetic diseases, inherited tumor syndromes and sporadic tumors. However, the molecular mechanism involved is still unclear.

The human *IGF2* gene is located on chromosome 11p15.5 and encodes a protein product of 67 amino acids functioning as a fetal growth factor and a cell mitogen. In fetal tissues, only the paternal allele is expressed while the maternal one is silent. The *H19* gene is located approximately 200 kb downstream of *IGF2*. In most of the tissues, only the maternal allele is expressed. The *H19* and *IGF2* genes are believed to be not only physically linked but also correlated by utilizing the same enhancer.

Among different forms of epigenetic modifications of DNA, methylation is believed to be the best candidate for the imprinting signal, and evidence has been shown that DNA methylation is involved in the regulation of both *IGF2* and *H19*.

In this study, promoter activities, general and promoter-specific imprinting, expression levels and DNA methylation status of the two genes were investigated in normal human livers and primary liver tumors, hepatoblastoma and hepatocellular carcinoma. Results show that the four promoters of *IGF2* are under a tight but dynamic control in a developmental-specific way. The P3 and P1 promoters are activated in fetal and adult livers respectively, each producing approximately 70% and 50% of the total *IGF2* transcripts. Methylation studies show that developmental and sequence-specific DNA methylation may be involved in the regulation of the promoter activities. The P2 and the P4 promoters are active throughout development with relatively minor changes in expression levels before and after birth. In adult, a relaxation of the imprinting from the P4 promoter can be seen by usage of the two parental alleles randomly. In both hepatoblastoma and hepatocellular carcinoma, promoter P1 is found significantly silenced and this turns out to be an important feature of liver tumors. P3 activity is upregulated in subgroups of both tumors with altered total expression levels of *IGF2* and *H19*. The loss of imprinting of *IGF2* can be found occasionally in both kinds of tumors.

In conclusion, the aberrant *IGF2* regulation, including dirupted promoter control by silencing of P1 and altered promoter-specific DNA methylation, is a frequent phenomenon in human liver cancers. Since the *IGF2* gene is regulated in a tight but dynamic, developmental-specific way, any disruption during this procedure may affect the normal liver development. Therefore, the molecular basis of the aberrant *IGF2* regulation is worth looking into to understand the molecular tumorigenesis of human liver cancers.

June 1997

Improving experimental tumor radioimmunotargeting

RAUN ROSSI NORRLUND

Department of Diagnostic Radiology, Umeå University, Umeå, Sweden

Radioimmunotargeting (RaIT) of tumors with labeled antibodies directed against tumor associated antigens has been used in about two decades. An experimental tumor model in athymic nude mouse was used to study the possibilities of improving RaIT. Nude mice, which are generally considered inert with regard to immune response against human tumors, were inoculated subcutaneously with HeLa Hep 2 cells. Monoclonal antibodies (mabs) TS1 against intracellular cytokeratin 8 and H7 against membrane bound placental alkaline phosphatase (PLAP) were used and labeled with ^{125}I. The animals were studied by repetitive quantitative planar radioimmunoscintigraphies and the absorbed doses to the tumors and whole body were calculated according to Medical Internal Radiation Dose (MIRD) Committee.

In order to improve tumor targeting the possibility of removing circulating antigens was investigated by preinjection of non-labeled idiotypic mabs. A postinjection of non-labeled anti-idiotypic mab was performed in order to remove non-targeted labeled idiotypic mabs.

The highest tumor to nontumor dose ratio by using anticytokeratin mab was achieved when a preinjection of non-labeled TS1 was combined with postinjection of anti-TS1 (αTS1). The dose ratio increased significantly compared to a single injection of ^{125}I-TS1, when four repeated tandem injections of ^{125}I-TS1 followed by αTS1 were administered in two weeks intervals. The highest mean tumor uptake was seen about three weeks after the injection of ^{125}I-labeled TS1 compared to about 4 days after injection of ^{125}I-labeled H7.

The highest tumor to nontumor dose ratio by using anti-PLAP was received after a single injection of ^{125}I-labeled H7. The dose ratio was not further improved by repetitive tandem injections of ^{125}I-labeled H7 and αH7. The dose ratio was not further improved by repetitive tandem injections of ^{125}I-labeled H7 and αH7.

The HeLa Hep 2 cell line was found to be immunogenic in nude mice by means of ELISA-, FACS-, BIAcore-evaluations as well as by immunofluorescence against both tested antigens, PLAP and cytokeratin 8. Predominantly, IgM antibodies were induced, but also IgG isotypes could be identified. Nude mice bearing human tumors produce significant amounts of antibodies against these two human tumor derived antigens. These endogenous antibodies may influence tumor targeting, by interfering with the pharmacokinetics of labeled or non-labeled idiotypic mabs at experimental RaIT.

In conclusion, RaIT was improved by a combination of preinjection of non-labeled TS1 and postinjection of its anti-idiotype and by repetitive tandem injections of ^{125}I-TS1 and its anti-idiotype. Using the same strategies the tumor dose increased by repetitive injections of H7 mab combined with its anti-idiotype, while the tumor to nontumor dose ratio decreased.

June 1997

The mode of action of the radiosensitizing analogs metoclopramide and nicotinamide

ANDERS OLSSON

Department of Cell and Molecular Biology, Section of Molecular Ecogenetics, Wallenberg Laboratory, Box 7031, S-220 07 Lund, Sweden

This thesis provides the first attempt to study the nicotinamides and benzamides from a mode of action point of view. Understanding the effects on DNA repair, cell cycle control (proliferation) and cell death from these agents and in combination with ionizing radiation, provides a new opportunity to logically develop better approaches to sensitize already existing therapies via for example induction of apoptosis or inhibition of DNA repair. Metoclopramide (MCA) and nicotinamide (NAM) have both been shown to possess radio-sensitizing properties. Their mode of action however seems to be quite different;

- MCA, a N-substituted benzamide sensitized; (i) Radiation induced DNA damage was sensitized *in vivo* when evaluated using three different methods, namely; alkaline elution, nucleoid sedimentation and measurements of the NAD pools, (ii) MCA sensitize low dose radiation in two different tumors; a human lung adeno carcinoma and a mouse sarcoma, carried by scid and CBA mice, respectively, (iii) MCA per se induces DNA damage, induces apoptosis, inhibits cell proliferation and clonogenicity *in vitro* in HL-60 cells and has a direct antitumor effect *in vivo*.
- NAM, a non-N-substituted benzamide analog; It was shown to have effects on (i) tumor vascularization, (ii) DNA repair, (iii) poly(ADP-ribosyl)-polymerase (PARP) inhibition, (iv) DNA damage, and (v) energy metabolism.

In addition, by conformationally altering the formulations of MCA pH adjustment, neutral MCA has become safer with less side effects without affecting its bioavailability and sensitizing properties.

In conclusion, the N-substituted benzamide analogs are distinguished from the non-N-substituted analogs because they are susceptible to radiolysis, induce cytotoxicity by apoptosis, inhibit cell proliferation and clonogenic growth, active PARP and have no effect on microregional tumor blood perfusion.

June 1997

Hereditary breast cancer in south Sweden—Early findings from studies on the role of BRCA1

OSKAR THOR JOHANNSSON

Jubileumsinstitutionen, Department of Oncology, Lund University, University Hospital, S-221 85 Lund, Sweden

The thesis presents the results from investigations into the role of BRCA1 in hereditary cancer in South Sweden.

Loss of heterozygosity (LOH) studies found loss of the wildtype allele of BRCA1 to be common in BRCA1 associated breast cancer, but due to the high degree of LOH on chr. 17q in sporadic breast cancer not to be indicative of the presence of a *BRCA1* mutation.

Seventeen different germline *BRCA1* mutations have been found in 34 separate breast and breast-ovarian cancer families. Five founder mutations were identified. If silent and suspected polymorphism mutations are excluded, frameshift, nonsense and splice mutations account for 93% in our material.

mRNA *in situ* hybridization of *BRCA1* was found to be able to identify BRCA1 and sporadic tumors with 95% specificity and sensitivity.

The histology and tumor biological features of *BRCA1* associated breast cancers was found to be predominantly of the ductal type, histological grade III, non-diploid with a high S-phase, predominantly *TP53* positive and *ER/PGR* negative tumors. Lymphocyte infiltration is a common feature in *BRCA1* breast cancer.

Comparative genomic hybridization (CGH) technique studies of DNA aberrations in BRCA1 tumors indicate that these have specific genomic changes in copy number (gains and losses) that are seldom seen in sporadic breast cancer.

On the role of the tumor suppressor gene p53 in leukemic cell differentiation

MATS EHINGER

Departments of Hematology, Research Department 2, E-block, University Hosptial, S-221 85 Lund, Sweden

Leukemic cells suffer from an impaired ability to differentiate due to inherited or acquired genetic lesions. These genetic changes can sometimes be bypassed with various compounds both *in vitro*, and, more rarely, *in vivo*, thus inducing terminal differentiation of the leukemic cells. Differentiation of both leukemic and normal hematopoietic cells is believed to be intimately coupled to cell cycle regulation and proliferation. The aim was therefore to increase the propensity of leukemic cells towards differentiation by overexpressing cell cycle active tumor suppresor genes, and to identify genes essential for differentiation pathways in these cells. The results show that the retinoblastoma protein, an important cell cycle regulating and tumor suppressor molecule, is necessary for induction of differentiation of certain leukemic cells with some agents. Furthermore, the mechanisms of differentiation do not merely depend on cell cycle regulation. The tumor suppressor protein p53 is another pivotal cell cycle regulator which halts the cell cycle in the G1-phase mainly by controlling the activity of the retinoblastoma protein. The present study demonstrates that it is possible to dispose leukemic cells for terminal differentiation by increasing the intracellular concentration of p53 through overexpression of the p53 gene. This was evidenced both by signs of terminal differentiation in response to p53 alone and by an increased sensitivity of p53-expressing cells towards a number of differentiation-inducing compounds. The mechanisms of p53-mediated differentiation do not necessarily rely on p53's ability to regulate the activity of the retinoblastoma protein and cell cycle progression. Furthermore, p53-independent terminal differentiation is achievable in growth arrested cells in the G1-phase of the cell cycle. G1-phase cell cycle arrest by itself does however not seem to be sufficient to induce any signs of differentiation. Therefore, it is proposed that differentiation and cell cycle regulation are two separately regulated processes.

September 1997

Neurofibromatosis type 1—Psychiatric and somatic aspects—A 12-year follow-up of adult patients in Sweden

MADELEINE ZÖLLER

Institute of Clinical Neuroscience, Department of Psychiatry, Sahlgrenska University Hospital Mölndal, S-431 80 Mölndal, Sweden

There is a shortage of follow-up studies of Neurofibromatosis type 1 (NF1), an autosomal dominantly inherited disorder. The population-based study in Göteborg in 1978 has been used for a 12-year follow-up on 70 NF1 patients. Our purpose was to study the impact of NF1 on psychiatric and somatic health.

Survival and life expectancy were estimated by using the Swedish population statistics. Clinical symptoms registered in the initial study in 1978 were analyzed in relation to mortality. Malignant and benign tumors were studied using the Swedish Cancer Registry. Medical records, death certificates and biopsies were examined. Heart rhythms were studied by a 12-lead ECG followed by Holter recordings.

The Comprehensive Psychopathological Rating Scale (CPRS) was used and psychiatric diseases were assessed by the Diagnostic and Statistical Manual of Mental Disorders 3rd ed. (DSM-III-R). Control persons were matched for age, sex and education. The Karolinska Scales of Personality (KSP) and the Self Evaluation Scale (SES) were used. Neuropsychology was studied by means of the Dureman Sälde Battery, the Halstead-Reitan Test Battery and the Benton Visual Retention Test.

The results show a considerably reduced life expectancy in the 70 NF1 patients and an increased mortality ($p < 0.001$). Mean age of living was reduced with about 15 years. There was a significantly increased number of malignant tumors reported in the NF1 cohort $p < 0.001$). Malignancy (55%) was the main reason for death followed by NF1 related complications (27%). Hypertension was associated with increased mortality. NF1 patients did not have any excess of arrhythmias.

NF1 was found to have a significant influence on psychiatric symptoms and personality variables. Mental illness was found in 1/3 of the patients. Dysthymia was the most common diagnosis. No significant progress was found after a 12-year period. The NF1 patients without psychiatric diagnosis outperformed the healthy controls in a positive self-evaluation. The NF1 patients displayed a variety of neuropsychological impairments.

The great impact of NF1 on psychiatric symptoms, neuropsychological functions and personality variables was a striking result. The many malignant tumors and the reduced life expectancy add to the psychological burden of NF1. Further long-term follow-up studies are needed.

October 1997

Characterisation of high energy bremsstrahlung beams

ANDERS SÄTHERBERG

Department of Radiation Physics, Umeå University, S-901 85 Umeå, Sweden

The photon beam from a new type of accelerator, called the racetrack microtron, has been studied. This beam is formed by a scanning technique which has the advantage of a small energy variation in a flat field, and it is also possible to create intensity modulated beams by changing the scan pattern. The Monte Carlo method has been used to study the photon beam from different target materials and configurations at 50 MV. Analytical calculations based on convolutions of scan patterns and Monte Carlo data are presented for flat and intensity modulated fields. Calculated half value layers in water are compared to measurements using two different set-ups and the calculated half value layers are in agreement with the measurements. However, a difference up to 5–6% between the two experimental set-ups was found. Analytical calculations of the absorbed dose distributions at 10 cm and 20 cm depth in water based on photon beams from linear accelerators taken from the literature are used for calculation of phantom scatter factors and TPR_{10}^{20}. The photon beam corresponds to a 4 MV and 24 MV linacc and to photon fields with the same energy distribution across the whole field. The different radial energy distributions did not affect the phantom scatter factors significantly, but the diagonal dose distributions, normalised at 10 cm, could, at 20 cm, differ by 4% at 80% of the diagonal. The heavy particle contribution to the absorbed dose in tissue at 50 MV has been investigated and the RBE was estimated as being between 1.01 and 1.03.

October 1997

BRCA1 patients do not appear to have a survival advantage compared to other breast cancer patients of the same age and stage, or to other breast and ovarian cancer in general.

June 1997

Radiology evaluation of bone marrow transplanted multiple myeloma patients

Brita Ågren

Department of Radiology, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden

This thesis concerns radiological evaluation of bone marrow transplanted multiple myeloma (MM) patients. The diagnostic radiological modalities evaluated are: conventional radiography (RG); bone scintigraphy (BS); bone marrow scintigraphy (MS); magnetic resonance imaging (MRI). The 242 included examinations are: 91 RG, 85 BS, 33 MS and 33 MRI. The 42 patients included have been treated with chemotherapy as well as with total body irradiation before bone-marrow transplantation.

In paper I RG is compared to BS with correlation to clinical data. The results showed that RG depicted more lesions than BS, while BS depicted fractures at sites not optimally seen on RG. RG and BS gave no information of prognostic value.

In paper II MS is compared to RG and BS. The results showed that MS only gave a marginal increase of information on focal lesions. MS was superior for depicting the sites of prior focal radiotherapy. MS showed that the majority of the patients had peripheral red bone marrow expansion. At MS, a monoclonal antibody radiopharmaceutical (which is specific for the granulopoietic system) was superior to a nanocolloid (which is specific for the reticulo-endothelial system) for the detection of lesions.

In paper III dynamic registration at BS and MS is evaluated. The results showed that no additional information was revealed at dynamic registrations when these were compared to static registrations or to RG and MRI.

In paper IV the location and cause of radionuclide activity noted occasionally in the abdominal region at MS is analysed. The conclusion was that the activity was localised to the gastro-intestinal tract and that the activity was considered to be at least partly due to an in vivo degradation of the radiopharmaceutical.

In paper V the use of MRI is evaluated. The results showed that a greater spread of lesions was found with MRI than with RG. For MM patients the optimal sequences were SE T1-weighted and TIR, performed without intra-venous contrast media.

The conclusions are:

– RG is the base for detecting lytic skeletal lesions
– BS is an auxiliary method for detecting suspected skeletal lesions
– MS depicts the entire distribution of red bone marrow
– MRI is the optimal method for depicting the entire patient, except for the bony skeleton. It has a high sensitivity but a low specificity.

September 1997

Diagnostic quality and absorbed dose in mammography—Influence of x-ray spectra and breast anatomy

Anne Thilander Klang

Department of Radiophysics, Göteborg University, Göteborg, Sweden

Optimisation in diagnostic radiology means that the radiation absorbed dose should be kept as low as is compatible with the image quality that is necessary for a correct diagnosis. In X-ray mammography, the image quality and the absorbed dose to the glandular tissue depend on the construction and settings of the X-ray unit, the thickness and composition of the individual breast and the properties of the image receptor used. The purpose was to give recommendations of which anode/filter and tube voltage combinations should be used for different breast thicknesses.

A large-area averaging optical densitometer (LAOD) was constructed and used to measure the mean optical density of a large area (16 cm^2) of the mammograms.

Among 1350 women with a mean age of 54.7 years from Göteborg, the average breast thickness was found to be 50 mm $\pm$ 13 mm (1 SD), which for dosimetric purpose corresponds to an equivalent thickness of polymethyl metacrylate (PMMA) of about 42 mm.

X-ray spectra obtained using different anode/filter combinations (Mo/Mo, Mo/Rh, Rh/Rh, Rh/Al and W/Rh) and tube voltages between 25 and 32 kV were measured using a Compton scattering spectrometer. Image quality studies were performed using an anthropomorphic and a physical phantom.

In a patient study including 965 women, the anode/filter and tube voltage combinations: Mo/Mo 26 kV, Mo/Rh 27 kV and W/Rh 26 kV were evaluated. The image contrast was highest in the Mo/Mo 26 kV mammograms. However, imaging of the glandular tissue, pectoral muscle and subcutis-skin was significantly ($P < 0.001$) better with the Mo/Rh 27 kV and the W/Rh 26 kV combinations than with the Mo/Mo 26 kV combination. The average mean glandular dose for W/Rh 26 kV and Mo/Rh 27 kV were 50% and 75%, respectively, of that for Mo/Mo 26 kV.

The difference in ability to detect (detection rate; the average fraction of objects detected in each image before the observer makes a false positive error) microcalcifications and opacities (simulating soft tissue lesions) was studied using an anthropomorphic breast phantom for the combinations Mo/Mo 26 kV and W/Rh 25 kV. The study also included an evaluation of image receptor (screen/film compared with storage phosphor plates) and a comparison of the influence of focal spot size and the use of a grid. The detection rate was in general higher for microcalcifications than for opacities, which were very low (18–38%) in the glandular tissue part of the phantom. The screen/film technique showed a significantly higher detection rate than the storage phosphor plates. No significant difference in overall detection rate was found between Mo/Mo 26 kV and W/Rh 25 kV.

Using Monte Carlo calculations, variation of contrast and signal-to-noise ratio (SNR) for breasts of different thicknesses (2, 4, 6, 8 and 10 cm) and compositions (1, 10, 25, 50 and 75% glandular tissue) was studied using measured X-ray spectra. Two pathological structures, a 5 mm thick tumour and a 200 μm calcification, were simulated. Two types of image receptors were also simulated: screen/film and digital technique. In screen/film imaging the contrast was highest for Mo/Mo combinations, but for thick breasts (≥ 8 cm) Mo/Rh, Rh/Rh and W/Rh are recommended, as they gave the same contrast with a lower dose than Mo/Mo. In digital imaging, for a constant image quality (SNR = 5), the dose was lower for Mo/Rh, Rh/Rh, W/Rh and R/Al than for the Mo/Mo combination for all breasts thicker than 2 cm.

Breast thickness is one of the most important parameters in the selection of an anode/filter and tube voltage combination. For screen/film mammography, Mo/Rh 27 kV is recommended for breast thicknesses of ≤ 60 mm and W/Rh 26 kV for breast > 60 mm.

In mammography, the optimisation of image quality must be focused on increasing the detectability of small invasive cancers (appearing as opacities only) in breasts with high proportion of glandular tissue.

September 1997