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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.

Assays for reverse transcriptase activity and p53 protein—Applications for characterisation of HIV RT mutants and inhibitors, and for p53 expression in breast cancer

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The work presented is focused on the development of methods for measurements of reverse transcriptase (RT) activity and p53 protein. The methods enable sensitive, simple and quantitative detection of proteins directly associated with the pathogenesis of human diseases, such as AIDS and cancer.

The first new technique developed for RT measurement was based on the use of a synthetic RNA (prA) as template, immobilised to a solid carrier, and a ^{125}I-labelled nucleotide as dNTP substrate. This improved both the technical performance and the assay sensitivity compared to the methods previously used. The carrier bound p constructs also facilitated the design of a method for combined affinity purification and RT assay. Furthermore, the properties of the new technique was instrumental for the design of methods for screening of RT inhibitors and evaluation of their mode of action. The sensitivity of the new assay was used to improve the techniques for detection of RT activity inhibiting antibody (RTab). This improved RTab-assay was then used to follow the titers of RTab in consecutively collected sera from a cohort of HIV-1 infected individuals. This study proved that decrease in RTab was a marker of disease progression and could be of prognostic value.

The other concept used for development of better RT assays lead to the design of a unique method for combined affinity purification and assay of RT. Monoclonal antibody (mab) with capacity to specifically bind HIV-1 RT without affecting its activity was immobilised on plastic beads. RT in crude samples is first attached to the immobilised mabs. Non-specific enzymes and other impurities were removed by a simple wash after which the RT reaction mixture was added. Substrate and product were finally separated by a wash of the beads. Practically all radioactivity incorporated into the DNA (>98%) was recovered on the bead. This assay had the capacity to recover approximately 80% of the RT activity added to an extract of 1×10^7 PBL cells/ml. The RT capture assay proved to be useful both for monitoring of HIV-1 infection in cell culture and for characterisation of the chain terminating capacity of RT from therapy resistant mutant virus.

Also presented is the development of a quantitative assay for the tumour suppressor gene product p53 in tumour samples from breast cancer patients. The method designed is a two site luminometric immunoassay (LIA), using two mabs for different epitopes at a non mutated site on p53. The assay is performed by incubating sample with a solution containing a luminescent tracer antibody in a plastic tube with solid phase antibody. After incubation, unbound tracer antibody is removed by saline washing. The amount of p53 is measured as the level of luminescence bound to the tube. This assay was found to have a very wide detection range. It also fulfilled the criteria for a routine assay. Its impact was evaluated in two clinical studies. A good concordance with the methods currently used was observed and the method also provided significant prognostic information.

1996

Molecular genetic studies of Ph-positive leukemias and the BCR and ABL genes

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In the present thesis molecular genetic methods were used to address different biological and clinical aspects of Philadelphia (Ph) chromosome-positive leukemias and the BCR and ABL genes. In the first study, children with Ph-positive chronic myeloid leukemia (CML) were found to have essentially the same breakpoint locations in BCR as adults, a pattern which differs from the one seen in Ph-positive acute lymphoid leukemia (ALL). In the second study, the breakpoint position within the major breakpoint cluster region (M-bcr) of the BCR gene was determined in 133 Ph-positive CML patients. No correlation was found between the breakpoint site and different clinical and/or hematologic parameters, including duration of the chronic phase and survival time. In the third study, the CRK proto-oncogene was localized to chromosome band 17p13, a segment frequently deleted during disease progression in CML, offering a new candidate gene to be investigated for its possible involvement in the disease progression. In the fourth study, it was shown that both BCR genes are expressed in the peripheral blood of healthy individuals, making it unlikely that imprinting would account for the previously reported parental-specific involvement of chromosomes 9 and 22 in the t(9;22). The identification of the adaptor protein CRKL as the major phosphorylated protein in the CML cell line K562 in the fifth study, and the demonstration that BCR/ABL and ABL bind to and phosphorylate CRKL, indicate that CRKL is likely to play a biologically important role in the development of Ph-positive leukemias. In the sixth study, first steps were taken to evaluate the effects of interferon-alfa (IFN-α) treatment on a transgenic mouse model for Ph-positive ALL. No prolonged survival or altered disease pattern was observed. In the final study, RNA in situ hybridization was used in an attempt to further elucidate the significance of the high expression of BCR in the brain. It was found that the expression of BCR was localized to highly specialized brain regions, reflecting a potentially interesting, yet presently poorly understood, function of BCR in the brain.

March 1996

Apoptosis in experimental and human prostate cancer—Significance in endocrone treatment and tumor progression

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Patients with prostate cancer (PC) commonly present with disseminated disease. For these patients, androgen ablation is a first line treatment. This mode of therapy usually has an initially palliative effect on tumor related symptoms and slows growth although virtually all tumors eventually relapse to an androgen independent, more aggressively growing phenotype. However, surprisingly little is known about the mechanisms mediating the initial palliative effect as well as the initiation of androgen inde-

pendent tumor growth. The present study was undertaken in order to further elucidate these matters.

Rats bearing androgen sensitive Dunning R3327PAP (DPAP) or G (DG) tumors were castrated and treatment effects were examined in tumors and normal prostates from the same animals. Increased numbers of apoptotic cells, increased expression of apoptosis markers including the apoptosis promoting Bcl-2-homologue Bax, and decreased cell numbers were detected in normal prostates but not in tumors. From PC patients, core biopsies were taken and treatment effects examined the day before and 7 days after castration therapy. A minority of PC patients responded to castration therapy with increased tumor apoptosis. However, the models as well as most PC patient tumors responded with decreased expression of proliferative markers as well as with other non-apoptotic regressive signs. This indicates that the initial therapeutic effects of castration therapy are primarily attributed to factors other than induction of apoptosis.

However, in DPAP tumors, combined castration and estrogen treatments resulted in a rapid volume reduction and an increase of the epithelial cell apoptotic index peaking at 24 hours of treatment. This cell death induction was preceded, at 12 hours of treatment, by an increased proliferative activity in the stroma. This indicates that combined castration and estrogen treatments can induce apoptosis in the DPAP tumor and that this induction may be mediated by estrogen-induced stromal activity.

On the other hand, castration alone rapidly reduced the apoptotic index in DG and DPAP tumors as well as in a subgroup of studied tumors in PC patients. In these and the other tumors in patients that did not respond with increased apoptosis, immunoreactivity for the anti apoptosis oncogene Bcl-2 was increased. These results indicate that in DPAP and DG tumors, a castration-induced rapid down regulation of tumor-cell apoptosis may be a major mechanism in the initiation of a more aggressive tumor phenotype leading to the eventual androgen independent progression and relapse of these tumors. This action, possibly mediated by an increased expression of Bcl-2, may also be an important contributing factor in the relapse for a majority of PC patients.

Moreover, 44% of the studied PC patients had pre castration Bcl-2 indexes over 10%, which corresponded to a non-apoptotic (including a decreased apoptotic index) response to castration therapy. This indicates the involvement of Bcl-2 overexpression in androgen-independent progression of PC following androgen ablation. In line with this, DG tumors engineered to express Bcl-2, in contrast to parental DG tumors, did not cease to grow or proliferate in response to castration therapy. This indicates that Bcl-2 expression confers an androgen independent phenotype to the originally androgen-sensitive DG tumor and may also contribute significantly to the development of an androgen independent phenotype in PC patients.

April 1996

Hematopoietic effects and clinical application of granulopoietic growth factors

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Granulopoietic growth factors (G-CSF and GM-CSF) stimulate the production of granulocytes and have multiple effects on mature granulocyte function. Both these hematopoietic growth factors are used in cancer patients to reduce toxicity of chemotherapy and to mobilise hematopoietic progenitor cells for transplantation.

The effect of GM-CSF on the regeneration of granulopoiesis after autologous bone marrow transplantation was studied by daily measurements of neutrophil granule proteins in 22 patients (GM-CSF 11, placebo 11). Despite a more rapid increase in absolute neutrophil count (ANC) in the GM-CSF group, there was no difference in blood levels of MPO. This suggests that GM-CSF increases ANC after ABMT by other mechanisms than by stimulating neutropoietic proliferation.

Neutrophil degranulation was studied in healthy volunteers receiving G-CSF once daily for six days. G-CSF enhanced the release of secondary granule proteins (lactoferrin, human neutrophil lipocalin). In vitro, G-CSF only marginally enhanced the release of these proteins, suggesting that the in vivo effect on secondary degranulation is indirect. G-CSF increased the plasma level of TNF-α.

Granulocyte function was studied in healthy volunteers receiving G-CSF once daily for six days. G-CSF impaired neutrophil chemotaxis, but enhanced neutrophil adherence and phagocytic capacity. G-CSF had no effect on the respiratory burst, as measured by lucigenin enhanced chemiluminescence.

Adult patients with newly diagnosed acute lymphoblastic leukaemia ($n = 80$) were randomised to receive either G-CSF or no G-CSF during 28 days remission induction chemotherapy. G-CSF reduced the duration of severe neutropenia, but had no impact on the incidence of infections, days with fever nor days with i.v. antibiotics.

Aiming to optimise the dose of G-CSF for mobilisation of hematopoietic progenitor cells in allogeneic donors, four groups of six male volunteers received G-CSF (lenograstim) at a dose of 3, 5, 7.5 or 10 μg/kg/d for six days. G-CSF was well tolerated. G-CSF 10 μg/kg/d produced a better mobilisation of progenitor cells compared to the lower doses. G-CSF favored mobilisation of primitive CD34+ subsets.

In an attempt to compare the efficacy of equivalent doses (μg) of lenograstim (glykosylated G-CSF) and non-glycosylated G-CSF (filgrastim) in mobilising progenitor cells (CD34+), healthy volunteers ($n = 32$) were randomised to receive either first lenograstim 10 μg/kg and then filgrastim 10 μg/kg or vice versa. A significant difference in favor of lenograstim was shown for peak number of CD34+ cells/μl blood. This finding indicates a difference in potency between the two G-CSFs.

April 1996

Regional cancer treatment—Experimental studies

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The survival in gastrointestinal malignancies has remained unchanged during the last decades. Regional cancer treatment can improve outcome compared to systemic treatment in some forms of cancer. The principal aim of the present work was to investigate different means of improving the efficacy and pharmacokinetics of regionally administered cytotoxic drugs.

A method of measuring the uptake of drug in the thoracic duct lymph and plasma after intraperitoneal (i.p.) instillation was developed in the pig in order to explore the pharmacokinetics of i.p. administration of three cytotoxic drugs, 5-fluorouracil (5-FU), carboplatin (CBDCA) and etoposide (VP-16). The effect of concomitant i.v. administration of vasopressin was determined. Total drug exposure (AUC) for 5-FU was in lymph six-fold greater than that in plasma after i.p. chemotherapy. Concurrent i.v. administration of vasopressin improved the pharmacokinetics of the i.p. route of administration of CBDCA and VP-16 by

two-fold. This may increase the activity of the drug in the abdominal cavity and in intraabdominal lymphatic tissue without increasing systemic toxicity.

In an experimental rat tumour model, the magnitude and duration of the decrease in energy charge in liver tumours after hepatic artery ligature (HAL) was determined. HAL gave rise to a transient energy depletion in the tumour which was not completely compensated for by glycolysis after one hour. The content of energy-rich nucleotides in the tumour was partially restored two hours after HAL. In the same tumour the influence of free radicals on the HAL-induced effect was determined. Desferrioxamine, a free radical scavenger, was administered in combination with HAL. Desferrioxamine had a tumour growth-retarding effect, both in vivo and in vitro in two experimental tumour systems and did not counteract the HAL-effect on the tumour.

The possibility of increasing the efficacy of regionally administered CBDCA by pretreatment with digitonin, a detergent that increases cell membrane permeability, was investigated in a rat tumour model. Regional pretreatment with digitonin via the hepatic artery increased the uptake of CBDCA administered via the same route by six-fold. The tumour growth-retarding effect of CBDCA was also potentiated.

April 1996

Pharmacokinetic and pharmacodynamic studies on cisplatin in mice and men

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Methodological tools for studies of the cytostatic agent cisplatin (CDDP) were explored and applied to elucidate various aspects of pharmacokinetics, drug distribution, chemomodulation and pharmacodynamics.

An immunohistochemical assay for analysis of CDDP-DNA adducts, i.e. the drug in its probable target position, was modified to allow quantitation with computerized image analysis. The methodological sources of error were estimated. We found the method to be feasible for comparing samples of the same tissue type, stained in the same batch and preferrably measured by one observer on one occasion.

The pharmacokinetics were studied as platinum (Pt) concentrations and CDDP-DNA adducts in nude mice. The highest tissue concentration was noted in kidney at 15 min. A biphasic elimination of Pt was observed in most sample types and the terminal half-life was similar (55 h–76 h) in whole-blood, serum, kidney, liver and testis. In brain the pharmacokinetics differed with a gradual accumulation during the study period of 7 days. Peak adduct levels were reached between 30 min and 4 h. Each tissue had its specific adduct staining pattern. With escalating CDDP doses there was a linear increase in both Pt concentrations and CDDP-DNA adducts in tissues including tumor. There were also good correlations between serum-Pt, tissue levels of Pt and adducts, respectively.

Heterogeneities in the intratumoral drug distribution were described and a model was presented for investigating the potential influence of vascularization and cell proliferation on intratumoral adduct distribution by using different immunohistochemical stainings of parallel tissue sections. A weak correlation was found between adducts and proliferation, which might indicate that drug uptake and adduct formation is increased in proliferating cells.

The antifungal agent amphotericin B was given to glioma-bearing rats with the purpose of enhancing the cytotoxicity of CDDP. The combined treatment resulted in excessive nephrotoxicity and in increased levels of CDDP-DNA adducts in kidneys. This indicates that nephrotoxicity is related to adduct formation of kidneys. It also shows that adduct analysis can be valuable tool for assessing the mechanisms of interaction between CDDP and modulating agents.

Ten patients were studied during the first cycle of CDDP-based chemotherapy. With limited-sampling and a population approach useful pharmacokinetic information was obtained. CDDP-DNA adducts in lymphocytes and buccal cells showed different kinetic profiles, possibly due to differences in cell turn-over. Renal damage, studied in terms of urinary protein excretion, was first displayed as tubular damage and later as impaired glomerular barrier function. Significant correlations were found between tubular dysfunction and pharmacokinetic parameters. These results could be the basis for further pharmacodynamic studies aiming towards individualized dose adaptation for cancer chemotherapy.

April 1996

Electron microscopy in diagnostic pathology with reference to mesenchymal tumors

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The experience acquired over a period of 15 years of diagnostic electron microscopy of fine needle aspirates and surgical specimens constitutes the base for this thesis. Some of the tumors analyzed represent rare soft tissue tumors, and consequently not very often ultrastructurally described. In locations where sarcomas are rare and unexpected, electron microscopy may contribute to a correct tumor classification when type specific structures are present.

Electron microscopic examination is a suitable method to confirm presence of mesenchymal tumor cells in cultures and to compare the morphology in cultures and fresh tumor tissue provided that the ultrastructural features are specific for the tumor in question.

Fine needle aspiration together with the use of electron microscopy is a reliable combination in the type-diagnosis of small round cell malignant tumors of childhood and adolescence and spindle cell tumors as peripheral nerve sheath tumors, leiomyosarcoma and synovial sarcoma, especially of the monophasic variant.

Electron microscopy contributes to the understanding of successive morphological changes in the dermal microvasculature of Kaposi's sarcoma.

This thesis presents examples that highlight the value of electron microscopy in the multidisciplinary management of musculoskeletal tumors.

April 1996

Apoptosis and histogenesis in neuroblastoma—Links to clinical heterogeneity

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Neuroblastoma is a malignant clonal expansion of cells that normally comprise the sympathetic nervous system. Clinical and biological variables that provide prognostic information for afflicted children suggest that normal tumor progenitor histogenesis, and the processes controlling cellular death, play a role in dictating tumor growth or regression.

Routine histology, immunohistochemistry, in situ hybridization, and a DNA fragmentation labeling technique (TUNEL) to identify apoptotic cells in situ, were employed in a selection of neuroblastoma tumors. Bcl-2 expression and the proportion of cells undergoing apoptosis were elevated in tumors procured from patients that experienced long-term survival versus non-survivors. As defined by the mitosis-karyorrhexis index, morphologically karyorrhectic cells were determined to be either proliferating (PNCA positive) or apoptotic.

A characteristic histologic arrangement of cells was observed in a prognostically favorable subset of neuroblastoma tumors. In these tumors, Bcl-2 reactive, proliferating tumor cells were identified in tumor regions adjacent to capillary-rich fibro-vascular stroma. As tumor cells gain distance from their blood-borne metabolic supply, they develop advanced morphologic and biochemical maturity, express neurotrophin receptors TrkA and TrkC, and cease proliferating. Those most differentiated tumor cells, which often express p53, p21, and Bax, but lack Bcl-2, lie adjacent to clusters of apoptotic cells. This tumor cellular organization suggests mechanisms by which tumor regression may occur.

Employing phenotypic marker gene expression profiles determined for tumors and normal tissues of the sympathetic nervous system, a comparative model of tumorigenesis and histogenesis was constructed. Sympathetic paraganglioma, pheochromocytoma, and stroma-rich neuroblastoma tumors possess marker expression profiles mimicking those of childhood sympathetic paraganglia, adrenal medullary chromaffin cells, and adrenal/extra-adrenal sympathetic neurons respectively. Favorable feature, differentiating neuroblastoma tumors possess a marker gene expression profile paralleling that observed for developing sympathetic paraganglia/SIF cells. In contrast, unfavorable feature neuroblastoma tumors mirror developmentally immature neuronal cells of sympathetic ganglia and the adrenal gland. These findings suggest that clinical features, such as primary tumor location and age at diagnosis, provide prognostic information for neuroblastoma patients by virtue of the existence and biology of the representative tumor progenitor cell type.

April 1996

Life change events and marital status in relation to risk and prognosis of cancer

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It is clear that a child's death is an extreme stressor for the parents. In this study, middle-aged women who have experienced the loss of a child did not have a risk and prognosis of cancer that was any different from women without this experience. Therefore, this result suggests that extreme psycho-social stress may not influence the risk and prognosis of cancer to any considerable extent.

Becoming divorced is a different type of stressor, and for divorced women, we found two striking patterns related to cancer risk. Firstly, this group had an increased risk of cancer of the lung and cervix, associations which, to a large extent may be due to a greater prevalence of smoking among divorced women. Secondly, divorced women were at lower risk of cancer at a number of sites (breast, endometrium, thyroid, hematologic malignancies, colon-rectum, malignant melanoma), compared to married women. These were unexpected results, that we are unable to explain using the current knowledge of potentially confounding factors. Instead, we need to pose new questions; in this group of divorced women, is it possible that certain factors could operate which selected women to divorce, and simultaneously, had an effect which lowered the risk of these cancers? Or, were women in the divorced group characterized by factors which somehow provided them with a protection against cancer? Our data can offer no answer to these questions, and they need to be studied in future research.

It is difficult to see that the results of our study can have any clinical implication. Nonetheless, many patients attribute their illness to emotional stress, and to them, these results may be of some consolation.

The null findings in our study correspond to a majority of the studies that we have cited, moreover, they agree with studies that we regard as the best in this area. The credibility of theories relating psychosocial events to cancer risk and prognosis may therefore be weakened by our results. Nonetheless, the risk patterns that we describe for divorced women remain unexplained and are not generally supported in the literature. Further studies on this aspect should be encouraged.

1996

The role of endogenous hypercholecystokiniemia and hypergastrinemia in growth and carcinogenesis of the exocrine pancreas

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The gut hormones cholecystokinin (CCK) and gastrin are phylogenetically and structurally related. Under normal conditions, CCK regulates growth of the exocrine pancreas and gastrin growth of the oxyntic mucosa of the stomach. The effects of chronically increased endogenous secretion of CCK or gastrin on pancreatic growth and carcinogenesis are not fully investigated. This thesis deals with such studies in rats and hamsters, using pancreaticobiliary diversion (PBD) to accomplish endogeneous hypercholecystokininemia and gastric fundectomy to accomplish endogenous hypergastrinemia.

PBD caused an immediate and persistent increase in the plasma CCK concentration without affecting the plasma gastrin concentration. In hamsters, PBD induced an initial hyperplasia (increased [^{3}H]-thymidine labeling index) of acinar cells followed by a persistent hypertrophy (increased weight, protein content, and DNA content) of the pancreas. No such changes were found in PBD operated hamsters receiving a CCK-A receptor antagonist. Longterm (14 months) PBD in rats lead to an increased fraction of aneuploid cells (DNA flow cytometry) in pancreatic tissue, and development of putative pre-neoplastic lesions (PPL) (acidophilic atypical acinar cell foci) and adenoma of the pancreas. No PPL or neoplasia were observed after longterm (8 months) PBD in hamsters. When rats were given a pancreatic carcinogen (azaserine), addition of PBD caused an increase in the fraction of aneuploid cells and in the volume fraction and cellular proliferation ([^{3}H]-thymidine labeling index) of the PPL. When hamsters were given a panceatic carcinogen (N-nitrosobis(2-oxopropyl)amine) (BOP), addition of PBD caused an increase in the incidence of PPL (ductal complexes) and ductal carcinoma in situ was observed. Furthermore, PBD caused an increase in the proliferative activity of the PPL. No increased proliferation in the PPL was seen in PBD operated animals receiving a CCK-A receptor angatonist.

Gastric fundectomy caused an immediate and persistent increase in the plasma gastrin concentration without affecting the plasma CCK concentration. In hamsters, fundectomy induced an initial hyperplasia of acinar, ductal, and centroacinar cells followed by a persistent hypertrophy of the pancreas. No such changes were found in fundectomized animals receiving a CCK-B/

gastrin receptor antagonist. Longterm (14 months) fundectomy in rats lead to an increase in the fraction of aneuploid cells in pancreatic tissue and development of PPL, but not neoplasia. No PPL or neoplasia were observed after long-standing (8 months) fundectomy in hamsters. When rats were given a pancreatic carcinogen (azaserine), addition of fundectomy lead to an increase in the fraction of aneuploid cells and an increase in the volume fraction, but not the proliferative activity, of the PPL. When hamsters were given a panceatic carcinogen (BOP), addition of fundectomy did not cause any significant change in the incidence or proliferative activity of PPL, and no neoplasia was observed.

In conclusion, PBD induced persistent hypercholecystokininemia and fundectomy persistent hypergastrinemia, both of which induced exocrine tissue hyperplasia leading to persistent hypertrophy of the pancreas in rats and hamsters. The effects of PBD and fundectomy seemed to be mediated by CCK and gastrin, since they were blocked by simultaneous administration of a specific CCK-A and CCK-B/gastrin receptor antagonist, respectively. In longterm studies, both procedures caused development of PPL of the pancreas in rats but not in hamsters. In both rats and hamsters treated with a pancreatic carcinogen, addition of PBD enhanced the development of PPL. In rats, but not in hamsters, addition of fundectomy to carcinogen treatment also enhanced the development of PPL. Overall, the trophic as well as the promotive effects were significantly more pronounced after PBD than after fundectomy. In both rats and hamsters, pancreatic neoplasia was observed after PBD, but not after fundectomy.

May 1996

Epidemiologic studies on the etiology of differentiated, non-medullary thyroid carcinoma

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The association between several risk factors and differentiated, non-medullary thyroid carcinoma (DNMTC) was studied in six population-based case-control studies conducted in the Uppsala Örebro Health Care Region of Sweden, including histologically confirmed cases of papillary and follicular thyroid carcinoma diagnosed January 1980 through September 1993.

The occurrence of cancer among parents of patients of DNMTC was compared with that of matched controls and with the whole population. An overall diagnosis of cancer in the parents was not associated with an increased risk of DNMTC. However, the incidence of cancer in the thyroid, nose and pharynx and of cancer in the stomach among mothers was higher than expected, while that in the breast and lung was lower.

The residential history of 484 cases of DNMTC and of as many controls was compared. The risk of cancer increased with residence over a long period in areas where goiter was endemic and, among women, if such a residence started at puberal age.

The risk of thyroid cancer in relation to parity was analyzed in a case-control study nested in a cohort of 1 700 000 women. A weak association was found with the number of livebirths among older women. Increasing age at the time of a livebirth was associated with an increased risk. During the first year after a livebirth the risk was twice as high uniparous as in nulliparous women.

In a questionnaire-based case-control study, conducted at the same time in Northern Norway and Sweden, no association was detected with pregnancies, oral contraceptives and hormone replacement after menopause. Pregnancy at an early age and artificial menopause were associated with an increased risk, and cigarette smoking with a decreased risk of DNMTC.

In the same study, the relation with diet was studied. High consumption of fat- and starch-rich food was associated with an increased risk of thyroid cancer. Goitrogenous vegetables increased the risk of thyroid cancer among residents in iodine-deficient, but not in iodine-rich areas.

The x-ray examinations administered to 484 cases and as many controls during their lifetime, prior to the date of diagnosis, were retrieved from the radiology archives of the hospitals in the regions where the subjects resided. The frequency of exposure to x-rays and the dose to the thyroid did not differ between the cases and the controls.

May 1996

Endometrial carcinoma—A descriptive and prognostic study with emphasis on proliferation, DNA ploidy and p53

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In a group of 384 women with endometrial carcinoma characterized by a medium or high risk of recurrence, 21 clinico-pathological variables were studied in the entire material or in subsets of patients. The aim was to assess the reproducibility of histopathological grading and to evaluate the prognostic impact of various variables, with emphasis on histopathological subtypes, nuclear grade, p53 protein expression, (flow cytometric) DNA ploidy and proliferation. As regards stages I–II, fourteen of 21 studied variables were prognostic in univariate analyses.

The reproducibility of the WHO, FIGO and nuclear grading systems was moderate, with a total agreement in about 60% of the cases. Despite this, all three systems, but especially nuclear grading, were also prognostic in multivariate Cox analyses. Uterine papillary serous carcinoma and clear cell carcinoma were also associated with a poor outcome in some of the multivariate analyses, but not in models in which nuclear grade or DNA ploidy were included.

p53 protein overexpression covaried with nuclear grade 3, aneuploidy and high SPF and was strongly prognostic in the univariate analysis and in multivariate models including age, stage and differentiation. It lost its independent prognostic impact when nuclear grade, DNA ploidy or SPF were included in the analysed models.

The three proliferation variables SPF, Ki67 and PCNA were significantly interrelated and all the variables were related to the degree of differentiation ($p < 0.05$). Aneuploidy was more common in tumours with strong Ki67 expression ($p < 0.01$) or high SPF ($p < 0.0001$). Ki67 was prognostic in univariate and some multivariate analyses whereas PCNA was not. SPF was, by far, the strongest predictor of survival.

Based on multivariate analyses, six prognostic index models based on 2 or 3 prognostically independent variables were constructed for various situations. These indices identified a large low-risk group (90% of the patients) with an estimated 5-year cancer-specific survival of 75–85%, depending on the index chosen, and a limited high-risk group (10% of the patients) with a 5-year survival of 30–52%. Prognostic index models were generally superior to the use of single variables for the assessment of prognosis.

In conclusion, more recent variables such as histopathological subtypes, nuclear grade, p53 protein overexpression and Ki67 were prognostic, but in multivariate analyses, aneuploidy and high SPF were the strongest predictors of survival (in the clinically important stages I–II). Treatment strategy (whether surgery be included or not) was also highly prognostic.

May 1996

Growth factor induced differentiation of cultured human neuroblastoma cells

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Neuroblastoma is a tumour of the sympathetic nervous system of children and infants. The neuroblastoma cell line SH-SY5Y has features of a differentiation-arrested progenitor cell of the sympathoadrenal cell lineage and has been the major model system in this study. Most growth factors tested affect these cells in a trophic and mitogenic manner. They also promote cell differentiation when combined with the phorbolester 12-O-tetradecanoylphorbol-13-acetate (TPA), similar to the already established and characterised sympathetic differentiation with TPA in the presence of fetal calf serum. However, a combination of the growth factors IGF-1 and bFGF, was able to promote differentiation in the absence of TPA. NGF, which plays a vital role during sympathetic neuronal differentiation and survival of the mature neurons, had no effect on SH-SY5Y. Therefore, TrkA was introduced by stable transfection, and cell clones that differentiated morphologically and functionally with NGF in a sympathetic neuronal manner NGF were isolated. The growth factor based differentiation protocols have been used to study the kinetics regarding the activation of the intracellular signalling proteins Shc, Raf and ERK, and RNA expression of c-fos. No distinct pattern of Ras-related signals and their differences in kinetics was seen by differentiating, or proliferating cultures. The differentiated SH-SY5Y phenotype caused by retinoic-acid treatment was also studied. These cells differ from the sympathetic nervous system phenotype, since they do not produce chatecholamines. These cells express the brain-derived neurotrophic factor (BDNF)-receptor (TrkB), despite that TrkB appears never to be expressed in sympathetic neurons or neuroblasts. The interest in characterising this phenotype came with the observation that bad-prognosis neuroblastomas with N-myc amplification tend to express both BDNF and TrkB.

May 1996

The study of eosinophil and monocyte cytotoxic activities in cancer

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Monocytes and eosinophil granulocytes infiltrate tumours of different origin. These two leukocytes have been shown to have cytotoxic effector functions vis à vis tumour cells. Both monocytes and eosinophils are able to generate reactive oxygen species and secrete other toxic substances.

The increased production of toxic oxygen metabolites observed in the whole blood of patients with cancer was explained by an increased activation of circulating blood monocytes. Monocytes were shown to exist in different subpopulations in blood with different capacities in oxidative activity. One subpopulation of monocytes with a higher level of activity was overrepresented in the blood of cancer patients. Another sign of increased monocyte activity in cancer was the increased serum level of TNF-α. The elevated monocyte activity is probably related to the cancer per se, since the production of reactive oxygen species and blood TNF-α concentrations slowly normalized, but with a delay of up to one year after tumour eradication.

The secretory activity of eosinophils was found to be increased in cancer, as demonstrated by elevated serum levels of the three granule proteins ECP, EPO and EPX. In contrast, however, the production of reactive oxygen species by the eosinophils was unaltered. One of the granule proteins, ECP, killed cancer cells in vitro. This capacity, however, was attributed to only one of several molecular variants of ECP and was inhibited by one monoclonal antibody, mAb 652, against ECP.

The results indicate that both eosinophils and monocytes are activated in cancer and that the body's defense against tumour cells involves the active participation of these two cells and their production of reactive oxygen species and secretion of cytotoxic proteins.

May 1996

Epidemiological studies on prostate cancer

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The aim of these studies was to investigate a wide range of possible risk factors for prostate cancer.

A population-based case-control study was conducted in the county of Örebro in 1989–1994. Comparisons of serum levels of pituitary and sex steroid hormones between a consecutive sample of 93 cases and 98 controls were made. We found no association between the serum levels of these hormones (obtained at diagnosis) and the risk of prostate cancer, nor did we observe any association between sex steroid hormone levels and the stage, grade or age of the patient.

A number of possible risk factors related to early life and adult life style were also investigated, based on information obtained through interviews of 256 cases and 252 controls. We found indications that childhood environment (urbanization) affects the risk of prostate cancer, and that pubertal events play a role. The impact of early diet appears small. Cigarette smoking and hard liquor consumption do not seem to be causally related to the risk of prostate cancer. A positive family history and a history of vasectomy were associated with an increased risk. Limited data also suggested that indices of high sexual activity implied an increased risk. A positive association between total energy intake and a risk was observed in a study based on 526 cases and 536 controls, where dietary information was obtained by self-administered questionnaires. The association was stronger for advanced prostate cancer. After adjustment for total energy intake no association was observed between any specific nutrient and the risk of prostate cancer.

In a large cohort study of 135 006 Swedish construction workers the association between various indices of body size as body mass index, height and lean body mass and incidence of and death from prostate cancer was investigated. All anthropometric measures were positively associated with the risk of prostate cancer, particularly the risk of developing fatal cancer.

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Improving radioimmunotrageting of tumors—The impact of extracorporeal immunoadsorption and perload in rats

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In radioimmunotherapy of tumors, uptake of the monoclonal antibody (MAb) used is often too low in relation to its uptake in normal tissues. The purpose of these studies was to improve experimental tumor radioimmunotargeting with (a) extracorpo-

real immunoadsorption (ECIA), where excess of radiolabeled MAbs circulating in blood is removed, (b) or by preload with unlabeled MAb prior to injection of radiolabeled MAb, (c) or by a combination of these. ECIA based on the avidin-biotin concept enables direct adsorption of radiolabeled and biotinylated MAb from blood and increases the tumor-to-normal tissue (T/N) uptake ratio by reducing background radioactivity in radiosensitive organs. 267 rats (athymic or Brown Norwegian) grafted with human adenocarcinoma or rat colon adenocarcinoma tumors intramuscularly, and beneath the kidney or liver capsule were included in the studies. Of these rats, 82 were subjected to ECIA. Two radioiodinated and biotinylated MAbs, murine L6 or chimeric BR96, were used and evaluated. (I) Using 50 μg dosage of L6, ECIA reduced whole body and plasma activity as well as improved the detectability of subrenal capsule tumors. T/N uptake ratios were increased on average 3 times. (II) The efficacy of ECIA in removing different injected amounts of L6 from plasma was similar. The highest T/N ratios persisting 24 h after start of the ECIA were obtained by using 10 μg of ^{125}I-L6-biotin. (III) The efficacy of preload in enhancing tumor uptake and simultaneously decreasing uptake in normal tissues was obtained with 250 μg of ^{125}I-L6 preceded by a preload of 50 μg unlabeled L6 only. (IV) The effects on radioimmunotargeting of preload and ECIA in combination were synergistic and improved T/N uptake ratios up to 17 times. (V) As compared with ECIA, a new method of whole blood immunoadsorption (WBIA) was technically easier to perform, safer and more reliable, but of approx. comparable efficiency. (VI) WBIA was even applicable on the internalizing and highly tumor selective ^{125}I-BR96-biotin MAb, resulting in manifestly improved T/N ratios.

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Primary liver cancer in Göteborg—Aspects on incidence, etiology and clinic

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Primary liver cancer (PLC) is one of the cancers with the highest reported increase of incidence rate in Sweden. The aim of the present study was to establish time trends of PLC in Göteborg, to identify probable etiological factors and to study various clinicopathological features and their relation to metastatic spread in hepatocellular carcinoma (HCC).

All cases diagnosed as PLC during a 22-year period with a high autopsy rate were reevaluated histopathologically and the case files were scrutinised. Time trends of PLC were calculated by use of an age-standardized incidence method and a Poisson model. The role of alcohol, hepatitis B (HBV) and C (HCV) virus in the etiology of HCC was also studied prospectively by interviews and by serological and immunohistochemical methods.

The majority (93%) of 711 cases registered as PLC were diagnosed histopathologically and in 98% specimens were available. Reevaluation revealed erroneous diagnosis in 9%. HCC constituted 90% and cholangiocellular carcinoma (CCC) 8% of all 590 PLC cases. The mean age for both HCC and CCC patients was 70 years. PLC was known or suspected only in 37% before death.

There was more than 50% increase of age-standardized incidence of PLC in Göteborg 1958–1979, but no significant increase was found, when using the Poisson model. The reason for this discrepancy is that the risk of HCC is extremely age-dependent and significantly higher than of all cancer in western Sweden ($p < 0.001$).

Both liver cirrhosis (72%) and alcoholism (21%) were more common in HCC compared with CCC ($p < 0.001$ and < 0.05, respectively). Cholelithiasis was more frequent (60%) in CCC than HCC ($p < 0.01$). Not a single case of chronic HBV infection was found in 40 HCC cases from 1976–1979 or in 63 cases examined prospectively. Seven of 64 HCC patients (11%) were anti-HCV positive. Cirrhosis was diagnosed in 69% of HCC cases, studied prospectively, with alcohol (42%) and HCV (19%) as the most common etiological factors.

Only five of 532 HCC patients in the retrospective material were cured. An autopsy was performed in 93% of the others. The most common sites of metastases were lymph nodes (42%), lungs (18%) and skeleton (17%). Features, significantly associated with metastases, were: increased liver mass, multinodular form, involvement of both lobes, low grade of differentiation, and vascular invasion.

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Role of mutations in the p53 gene for the development of human squamous cell skin cancer—A correlative molecular and morphological study

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Mutations in the p53 gene are the most frequent somatic genetic alterations in human cancer. Ultraviolet light is etiologically associated with squamous cell cancer of the skin and its precursors. The importance of mutations in the p53 gene for UV induced squamous cell cancer of the skin is unknown.

The acute reaction of normal healthy epidermis to UV irradiation was monitored by following the accumulation of p53 and p21 by immunohistochemistry. Both proteins began to accumulate after a few hours to reach a peak at about 24 hours and return to low base values within 4–7 days. Chronic reactions to UV irradiation were examined in 425 sections of normal skin adjacent to a variety of neoplastic/non-neoplastic lesions from stored tissue blocks. A dispersed pattern of p53 accumulation similar to the acute response was noted.

It was discovered that morphologically normal epidermis contained sharply demarcated patches with strong accumulation of p53. To reconstruct the two dimensional distribution of patches, 1 320 consecutive sections were examined from 11 excisions of human skin with different lesions. 40 patches were recorded. Their size varied from 0.03 to 12 mm^2. It was calculated that there are about 20 p53 gene mutations/cm^2. This suggests that the prevalence of p53 gene mutations is about 1:30 000 stem cells in normal sun-exposed skin. The patches were rarely in contact with lesions. This compact pattern of positive immunohistochemistry was similar to that seen in cancer/precancer.

To elucidate the genetic relation between patches and cancer/precancer, microdissection was performed. DNA was sequenced for mutations in exons 4 or 5 to 8 of the p53 gene supplemented by analysis of LOH. 133 samples from 24 patients with different lesions including normal epidermis were analyzed. In simultaneously present cancer/precancers at least one mutation was identical. This strongly indicates that squamous cell cancer and its precursors are clonal neoplasias. The data suggest that mutation of the p53 gene is an early event in the development of those squamous cell skin cancers which carry these mutations (about 50%).

About 70% of the patches also carried mutations in the p53 gene, but these were never the same as those of the adjacent cancer/precancer. It is suggested that p53 mutations in these clonally expanded keratinocytes are innocuous and that their

eventual precancerous potential is very small. The mutation spectrum of patches and cancer/precancer were similar. No LOH was found in the patches but 37% of cancer/precancer had LOH.

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Radiotherapy of prostatic adenocarcinoma—with reference to local cure, characterization of residual tumour cells and assessment of adverse effects

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External beam radiotherapy (RRT) of prostatic adenocarcinoma can achieve a clinical local control as assessed by digital rectal examination (DRE) in a high proportion of patients. However, the extent and the clinical significance of histopathologically demonstrated residual tumour cells after RRT is debated. The present study investigated 55 patients, the majority of whom had obtained clinical local control after an average follow-up of 6.8 years post RRT. The extent of histopathological tumour eradication was assessed in systematic core biopsies (CB) obtained with the guidance of transrectal ultrasonography (TRUS). Residual tumour cells were demonstrated in 67% of the patients. The sensitivity of DRE and TRUS was low. RRT causes severe morphological changes in cells, which sometimes lose their epithelial character. The biological characteristics of residual tumour cells are unknown and immunohistochemical stainings were performed to obtain further information.

Ki-67 and PCNA antigens were expressed in 64% and 94% of residual tumours, respectively, indicating a retained proliferative capacity of the cells after RRT. Tumours also retained their epithelial characteristics, as demonstrated by 97% positivity for the epithelial marker cytokeratin 8 (CK8). Prostate-specific antigen (PSA) and Prostatic acidic phosphatase (PAP) were observed in 94% and 81% of the tumours, indicating a retained biochemical capacity. Alterations in the p53 tumour suppressor function, as measured by p53 nuclear protein accumulation, was demonstrated in a low proportion of cases (11%). However, the cell cycle inhibitory protein, p21, that exerts control mechanisms during the late G1-phase, was expressed in only 47% of the tumours, indicating that a substantial proportion of tumour cells manage to escape this check-point control and continue to propagate in the cell cycle. The results suggest that the malignant potential of these residual cells is maintained.

A higher proportion of distant metastases and an increased risk of disease-specific deaths was demonstrated in patient with residual local tumour, as compared with biopsy-proven tumour-free patients at a 2-year post-biopsy follow-up.

Adverse effects were assessed in 66 patients. Sexual functions were substantially impaired in 60%. Late bowel and urinary adverse effects that affected individual life-styles, occurred in 3% and 6%, respectively.

The results form a basis for decisions on further developments of radiotherapy techniques to achieve a higher rate of tumour eradication without enhancing, rather minimizing, morbidity.

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Proteolytic mechanisms in tumor spread—Studies on the role of serine proteinases in human gastrointestinal cancer

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Alterations within the proteinase cascade systems and increased extracellular proteolysis appear to be hallmarks of aggressive malignancy. We conclude that proteolytic mechanisms, involving serine proteinase cascade systems, participate in the local invasion of solid tumor cells and in the accumulation of malignancy-related ascites in gastrointestinal cancer.

Serine proteinases and proteolytic mechanisms in the invasion of solid tumor cells. uPA and uPA receptor are accumulated at the tumor-host interface and PAI-1 within the tumor epithelium and stroma of human colorectal adenocarcinomas. Both human colon carcinoma cells and fibroblasts express PAs and PAIs, whereas only the tumor cells express the uPA receptor required for focalization and regulation of the cell surface PA activity. While uPA and uPA receptor at the invading front can participate in tissue degradation and tumor invasion by plasmin generation, PAI-1 in the tumor tissue may protect the tumor itself against proteolytic degradation.

Serine proteinases as prognostic markers in solid tumors. The antigen levels of uPA and uPA receptor at the tumor-host interface are related to the tumor aggressiveness of colorectal adenocarcinomas and may be useful prognostic markers in colorectal cancer. In Dukes' stage C tumors with other poor prognostic markers, the uPA level was twice the level in Dukes' stage A tumors with good prognostic markers. Both the uPA level and the uPA receptor level were significantly higher in the Dukes' stage C tumors, while the PAI-1 level was not significantly different from the Dukes' stage A tumors.

Serine proteinases and proteolytic mechanisms in the accumulation of ascites. The fibrinolytic, contact activation, and kallikrein-kinin systems are all activated in ascites from patients with gastrointestinal malignancies. Intraperitoneal generation of bradykinin and other mediators, due to activation of these proteinase cascade systems, can be of importance for the continuous generation of ascites in gastrointestinal cancer.

Serine proteinases and inhibitors as diagnostic markers in ascites. Serine proteinase inhibitors of the contact activation and kallikrein-kinin systems, α_2-macro-globulin, C1-inhibitor, and α_1-proteinase inhibitor, and total proteins are valuable parameters for the differential diagnosis of human ascites. Serine proteinases of the fibrinolytic system, plasmin and tPA, may also be useful parameters for the differential diagnosis of ascites.

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