Acta Oncologica Vol. 32, No. 6, pp. 694–696, 1993

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.

Endometrial carcinoma—Steroid hormones and receptors in relation to proliferation

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The significance of the hormonal milieu for endometrial changes is as well-known as its link with endometrial carcinoma. Unopposed oestradiol treatment is shown to increase the incidence for this cancer. Obesity leads to elevated levels of oestrogens and is a risk factor for endometrial carcinoma. An association between high tumor proliferation and prognosis is a general feature of human cancer. Tumour growth can be expressed as proliferation rate and flow cytometry (FCM) is a sensitive and reproducable method to estimate S-phase fraction (SPF) and ploidy level. Both parameters have been shown to correlate with prognisis. Sex steroid hormone levels were analysed together with clinical parameters, SPF, and receptors in established endometrial carcinoma. The study consisted of postmenopausal women with endometrial adenocarcinoma. Hormones were analysed in 127 patients, 99 were analysed for FCM and 60 for oestrogen and progesterone receptors. RIA technique was used for hormone assay of oestrone, oestradiol, progesterone, androstenedione and testosterone plasma levels. The receptors were analysed with an immunohistochemical method, and SPF and ploidy level by flow cytometry. A wide range of oestrogen concentrations was found. Some patients had levels comparable to fertile women. Strong correlations were found between body mass index, weight and depth of uterine cavity. No relations were found between receptors and SPF, apart from oestrogen-receptor positive tumours having a lower SPF when compared with receptor negative tumours. The influence of oestradiol on tumour proliferation expressed as SPF was ambiguous. SPF was increased with higher oestradiol levels in the group of peri-diploid, well-differentiated tumours, while a negative correlation was found for the peri-diploid, moderately differentiated tumours. The aneuploid and poorly differentiated tumours had a high SPF regardless of oestradiol concentration. The association between progesterone concentration and SPF was of a more general nature. Progesterone above a threshold level was related to a lower SPF in well-differentiated and moderately differentiated tumours. Thus endogenous progesterone seems to play a role in controlling the tumour's proliferation activity, in contrast to oestradiol, that had a role which did not appear to relate to proliferation activity in any specific direction. The only stimulative association was seen in well-differentiated tumours, but SPF was still below the mean value for all diploid tumours.

June 1993

Oncoprotein 18—Junction where receptor and cell cycle regulated protein kinase systems interact

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The present thesis suggests a putative role of Oncoprotein 18 (Op18) in signal transduction and growth control. Evidence for such a role of Op18 includes: a) phosphorylation of Op18 in response to stimulation of the antigen receptor of T lymphocytes b) cell cycle regulation of Op18 phosphorylation c) differentiation specific regulation of Op18 expression levels, and d) profound up-regulation of Op18 in malignant lymphoma/leukemia cells. Analysis of Op18 suggests a very complex pattern of phosphorylation, and the site mapping studies performed in this thesis provides some clues that are likely to be central in the search for the function(s) of this phosphoprotein. Two distinct proline directed kinase families have been identified that phosphorylate Op18 with overlapping but distinct site preference. These two kinase families, mitogen activated protein (MAP) kinases and cyclin dependent cdc2 kinases, are involved in T lymphocyte antigen receptor and cell cycle regulated phosphorylation events, respectively. Site mappong studies performed in vivo and in vitro have shown that MAP kinase has a 20-fold preference for Ser25 as opposed to Ser38 of Op18, while cdc2 kinases have a 5-fold preference for the Ser38 residue. In addition to phosphorylation of Ser25 and 38, a calcium induced phosphorylation event has been identified that results in phosphorylation of Ser16 of Op18. In conclusion, the result shows that multiple potentially growth regulatory protein kinase pathways induce phosphorylation of Op18 Ser16, 25 and 38 in T lymphocytes. In parallel to studies of Op18 phosphorylation in response to antigen receptor stimulation of T lymphocytes, site mapping studies of cell cycle regulated fluctuation of Op18 phosphorylation was performed. The results reveal that S-phase progression of a synchronised leukemic T cell line is associated with increased phosphorylation of both the Ser25 and 38 residues. Moreover, during mitosis Op18 is stoichiometrically phosphorylated at Ser38 resulting in a cellular pool of Op18 where approx. 50 percent of all Op18 molecules are di-phosphorylated on both Ser25 and Ser38. In vitro phosphorylation experiments, employing two distinct members of the cdc2 kinase family, suggest involvement of both p34-cdc and CDK2 in cell cycle regulated phosphorylation of Ser25 and 38 of Op18. Thus, Op18 seems to be a major substrate for members of the cdc2 kinase family during both the S-phase and the mitotic phase of the cell cycle. The result outlined above suggest that the Op18 protein may reside at a junction where receptor and cell cycle regulated protein kinases interacts. Estabilishment of transfected cell lines that overexpressed Op18 resulted in a somewhat surprising finding. The initial expectation, based on overexpression of Op18 in lymphoid malignancies, was that ectopic expression of Op18 in a non-transformed cell line would result in less stringent growth control. However, the result showed that overexpression of Op18 results in a partial growth arrest. It seems possible that the phenotypic outcome of ectopic expression of Op18 is governed by the phosphorylation status of this cytoplasmic protein. As outlined above, distinct Op18 Ser residues are targets for MAP kinases, cdc2 kinases and a calcium regulated kinase(s). The identification of Ser16, 25, and 38 as targets for distinct kinase systems provide the basis for experiments where the putative function(s) of the observed phosphorylation events may be elucidated.

September 1993

Colorectal cancer and non-malignant respiratory disease in asbestos cement and cement workers—Studies on mortality, cancer morbidity, and radiographical changes in lung parenchyma and pleura

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Radiologically visible parenchymal changes (small opacities $\geq 1/0$; ILO 1980 classification) were present in 20% of a sample of

workers (n = 174), employed for 20 years (median) in an asbestos cement plant. Exposure-response relationships were found, after controlling for age and smoking habits. Workers with high average intensity of exposure had a higher risk for progression of parenchymal changes after end of employment, and experienced a higher risk for costophrenic angle obliteration than those with low exposure intensity. In a sample of asbestos cement workers with symptoms and signs suggestive of pulmonary disease (n = 33), increased lung density measured by x-ray computed tomography, and reduced static lung volumes and lung compliance was found. In a cohort of asbestos cement workers (n = 1 929) with an estimated median exposure of 1.2 fibres/ml, the mortality from non-malignant respiratory disease was increased in comparison to a regional reference cohort (n = 1 233). A two- to three-fold increase of non-malignant respiratory mortality was noted among workers employed for more than a decade in the asbestos cement plant, compared to cement workers (n = 1 526), who in their turn did not experience an increased risk compared to the general population. In the cohorts of asbestos cement and cement workers, there was a two- to three-fold increased incidence of cancer in the right part of the colon, compared to the general population as well as to external reference cohorts of other industrial workers (n = 3 965) and fishermen (n = 8 092). A causal relation with the exposure to mineral dust and fibres was supported by the findings of higher risk estmates in subgroups with high cumulated asbestos doses or longer duration of cement work. The incidence of cancer in the left part of the colon was not increased. Morbidity data, but not mortality data, disclosed the subsite-specific risk pattern. Both asbestos cement workers and cement workers had an increased incidence of rectal cancer, compared with the general population, and with the fishermen. The risk was, however, of the same magnitude among the other industrial workers.

September 1993

Colonic healing—The effect of irradiation and chemotherapy—An experimental study, resembling adjuvant therapy for colorectal carcinoma

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Adjuvant treatment of colon and rectal carcinoma is of major interest. Irradiation and chemotherapy are modalities used widely. Concern for the integrity of anastomoses performed when this treatment is used has been put forth. The purpose of this study was to evaluate the effect of preoperative irradiation and postoperative intraperitoneal 5-fluorouracil treatment on colonic healing. In rats preoperative irradiation of the lower abdominal region by 10 + 10 Gy four days apart caused inflammatory reaction in the colon as evaluated by histology and determination of myeloperoxidase activity. The inflammatory reaction reached its peak within a week of the second irradiation. When standardised colonic resections and anastomoses were performed within the irradiated part of the colon the anastomotic healing was not affected during the first week after operation as judged by complications and breaking strength. A lower breaking strength and an increase in myeloperoxidase activity two months after operation may indicate late changes within the intestinal wall. Intraperitoneal 5-fluorouracil in rat given immediately after colonic resection and repeated as daily injections caused a weight loss and a marked reduction in breaking strength of the anastomosis as well as in the abdominal skin wound. The addition of intravenous leucovorin did not further impair wound healing. A reduction in 5-fluorouracil concentration did not alter the negative wound healing effect of the chemotherapy. In a group of rats subjected to nutritional depletion, mimicking the weight curve of 5-fluorouracil treated animals, anastomotic breaking strength was not compromised to the same extent as when 5-fluorouracil was given. This indicates a direct toxic effect rather than an effect of reduced food intake caused by 5-FU treatment. Collagen synthesis and the formation of new tissue in the wound gap was reduced in 5-fluorouracil treated animals compared to controls as judged by in vivo incorporation of ^{3}H-proline in the anastomotic segment and determination of anastomotic breaking strength after removal of sutures. When administration of 5-fluorouracil was postponed by three days the negative wound healing effects of 5-FU were eliminated. The results indicate an early vulnerable phase in which 5-fluorouracil may have a detrimental effect on colonic healing.

Serptember 1993

Localized prostatic cancer—A study of diagnosis, staging and prognostic factors

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Carcinoma of the prostate is the most common malignancy in Swedish males. Current methods for patient selection lack the necessary sensitivity and specificity for selecting those for whom cure is required and possible. The aims of the present study were to: 1) characterize the tumour morphology postoperatively with the necessary thoroughness for comparison of preoperative diagnostic efforts to the former characteristics, 2) assess the ability of the current standard preoperative diagnostic biopsy effort to predict these characteristics, 3) compare these pre- and postoperative findings with the clinical outcome, and 4) establish the necessary amount of biopsy material needed for an accurate characterization of localized prostatic cancer. One hundred and forty-one consecutive radically prostatectomized patients were evaluated, the first 43 with a limited assessment of their surgical specimens, 98 with an extensive serial step-sectioning technique. The morphological (tumour grade, presence of prostatic intraepithelial neoplasia /PIN/, distribution, volume, pathological stage) as well as cellular (DNA ploidy) characteristics were assessed in the latter series. Preoperative biopsies were assessed for tumour grade and DNA ploidy. The last 60 cases also underwent an extensive 10 biopsy mapping procedure in vitro. The limited assessment of the first 43 operative specimens was not sufficient for characterization of the disease. Extensive serial step-sectioning is necessary, in which the tumours are shown to be multifocal, merging into larger tumour foci with increasing volume, intermingled with PIN, whose relative volume decreases with increasing cancer volume, heterogenous both regarding grade and DNA ploidy. Non-diploid DNA when present, always in the largest tumour focus, correlated to larger tumour volumes and was a strong predictor of capsular penetration. The current preoperative standard 1–3 diagnostic biopsies do not suffice to achieve an accurate assessment of either the extent, distribution, grade or DNA ploidy of the malignancy, when compared to the serially step sectioned specimens. Furthermore, prognostic factors, significant for the 3-year outcome, cannot be correctly predicted. An in vitro mapping study of the last 60 specimens revealed that mapping with 6–10 biopsies from all parts of the gland can achieve this and be feasible in vivo.

October 1993

Assessment of cell heterogeneity in drug response

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The development of the individual colony formation assay (iCFA) is described. The iCFA, using automated image analysis, permits the tracking of growth at the individual colony level. In principle, this will permit quantitative characterization of cellular heterogeneity in growth rate and cellular heterogeneity in response to proliferation-modifying agents. In addition, growth rate can be related to other parameters e.g. metabolic viability. A human colon cancer cell line (HCT-8) was used when the basic characteristics of the iCFA was explored and when a clone, 1 000-fold resistant for FdUrd, was selected based on growth rate. The FdUrd resistant clone was shown to be deficient in thymidine kinase but exhibited greater sensitivity to 5-FU, the TS inhibitor ICI D1694, and to methotrexate than the parent cell line. When the response of the HCT-8 cell line to 5-fluoro-2'-deoxyuridine (FdUrd) was evaluated it was found that in the presence of a drug concentration which, in standard monolayer assays, inhibits the growth to about 50% (IC_{50}) only about 20% of the colonies ceased to grow and the remaining colonies grew at a growth rate of about 70% of control. At an FdUrd concentration which, in standard monolayer assays, reduced growth by >90% (IC_{90}), about 50% of the cells grew, with growth rates of about 30% of control. When a human leukemia cell line (K-562) was exposed to doxorubicin, daunorubicin, or idarubicin and a mouse leukemia cell line (L-1210) was exposed to araC, three distinct growth patterns emerged. Cells could be categorized as continuous growing at a decreased growth rate, limited growth, or no growth. These surviving, slowly growing cells, may represent a previously unrecognized population that may contribute to therapeutic failure.

October 1993

Psychosocial adjustment after breast cancer in stages I/II—A longitudinal study including assessments of women and husbands

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In a quasi-experimental study, the impact of type of breast cancer surgery on postoperative psychosocial adjustment was investigated in 99 women treated for a breast cancer in stages I/II. Semistructured interviews based on the Social Adjustment Scale (SAS) were conducted 4 and 13 months after surgery. Thirty-seven women had breast-conserving surgery (BCT) and 62 women had a mastectomy (MT). A total of 56 husbands participated in the study and were interviewed separately with Social Behaviour Assessment Schedule (SBAS) at the same points in time. A long-term follow-up (range 5.8–8.1 years) included 66 women (BCT n = 26, MT n = 40). There were no statistically significant differences between the two groups of women at 4 months or in the follow-up after 6 years with respect to their psychosocial adjustment. However, at 13 months, the adjustment was better in the BCT-group. In analyses of risk factors for maladjustment, the type of surgery (4 mo: OR 4.95, 95% confidence interval: 1.1–23.4/13 mo: OR 5.52, 95% confidence interval: 1.1–27.1) and radiotherapy (4 mo: OR 3.97, 95% confidence interval: 1.01–15.7) were found to increase the risk for maladjustment. Among the participating husbands, 48% expressed feelings of distress at some time during the investigation. Husbands in the MT-group had more depression at 4 months ($p<0.05$) than did those in the BCT-group. The agreement was found to be high ($r = 0.74$) in the couples' assessment of the experience of a breast cancer diagnosis and treatment. The marital relation was found to be a strong predictor of the women's psychosocial outcome at 13 months ($R^2 = 0.53$). In the long-term follow-up, 10% of the women in the sample expressed feelings of psychosocial maladjustment related to their breast cancer. No association was found between the psychosocial assessments from 4 and 13 months and overall or recurrence-free survival, nor with the civil status or age of the women.

October 1993

Radiation quality dependent factors in dosimetry and their correlation to photon and electron beam characteristics

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The subject of this dissertation is the analysis of the different steps involved in the dosimetry chain, from the calibration of the dosimeter to the estimation of the dose and dose distribution inside the patient irradiated by high-energy electron and photon beams. Special attention is paid to the radiation quality dependent factors which influence gas filled ionization chamber dosimetry and the problems involved in assigning an index of quality to a beam. For photon beams an approach based on narrow beam water penetration properties is developed; the method avoids a number of shortcomings, particularly those at higher energies, which were associated with earlier methods. Calibration procedures of plane-parallel ionization chambers in ^{60}Co γ-ray beams are proposed, and necessary correction factors are given for a number of commercially available plane-parallel chambers. The behaviour of one type of chamber, the NACP chamber, in photon beams over a wide range of energies, is studied with Fricke dosimetry as a reference. The properties of liquid ionization chambers are investigated with respect to energy dependence and, due to their favourable energy characteristics, the chambers are used to determine the stopping power ratio water-to-air in photon beams. These ratios are then correlated to the narrow beam penetration properties of the beams. The beam characteristics and special features of the MM50 race-track microtron are thoroughly investigated, both for electron and photon beams, for a number of energies up to 50 MeV and 50 MV, respectively. It is shown that the special design characteristics, involving field flattening by a scanning beam technique and minimization of scattering material in the treatment head, can be used to improve the dose distributions for both photon and electron beams. Changes in the biological effect due to the high pulse doses used in scanned electron fields are shown to be insignificant.

October 1993