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.

Glycoconjugates in human meningiomas—The search for tissue and circulating tumor markers using ligand binding techniques

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The purpose of this investigation was to define tumor-associated glycoconjugates in meningioma tissues and to investigate whether tumor associated structures can be markers for meningiomas in CSF using specific monoclonal antibodies, toxins and lectins in immunostaining procedures. To achieve these purposes the following methods have been developed.

Lectin-blotting methods for the detection of tumor associated glycoproteins have been optimized. The glycoproteins were resolved and blotted in two different ways 1) conventional SDS-PAGE and electrophoretical blotting and 2) SDS-PAGE and thermoblotting on the PhastSystem(TM) (Pharmacia). The PhastSystem showed several advantages; new protein bands were detected in the low molecular weight region, the resolution was high, less protein was needed and the analysis time was reduced.

To determine glycolipids in individual CSF samples a micro-immunoaffinity technique including extraction, purification and enzyme/TLC immunostaining was developed. Gangliosides of the gangliotetraose series (GM1, GD1a, GD1b and GT1b) were individually determined with cholera toxin B-subunit in TLC-ELISA after chromatography and sialidase hydrolysis to GM1. The lower detection limit for each ganglioside was 100 fmol/l. Other ganglioside and sulphatide were detected by specific MAbs in TLC-ELISA.

The ganglioside composition has been investigated in 60 meningiomas. The major gangliosides were GM3 and GD3 and most of the meningiomas could be separated into two groups, a GM3-rich (38%) and a GD3-rich group (32%), based on the proportion of mono and oligosialogangliosides and the content of GM3 and GD3. The meningiomas which had in between values were placed in a third, intermediate group. There was no correlation between these ganglioside groups and the histopathology. The expression of GD3 was found to be associated with monosomy of chromosome 22, occurring in approximately 50% of meningiomas. The probability of predicting monosomy from the GD3 proportion was 75%.

Tumor-associated glycoproteins were also detected by the lectins RCA, PNA and WGA in meningioma tissue specimens.

As compared to the control groups the CSF samples from meningioma patients were found to contain increased amounts of GD3. Also tumor associated glycoproteins detected by the RCA lectin in meningioma specimens were found in the CSF from the patients. These results support the idea of shedding from the meningioma cells to CSF.

In conclusion, this study indicates that there are both gangliosides and glycoproteins which might be valuable tools for the early diagnosis of meningiomas. To evaluate their usefulness an extended study, including a larger number of patients is under way.

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Soft tissue tumors with Schwann cell and melanocytic differentiation—Studies of peripheral nerve sheath tumors, clear cell sarcoma and sarcoma-like malignant melanoma

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The clinicopathologic, electronmicroscopic and immunohistochemical properties of 15 clear cell sarcomas of tendons and aponeuroses (CCS), 14 malignant epithelioid schwannomas (EMS) of peripheral nerve sheath, 29 cellular schwannomas (CS) and 21 soft tissue sarcoma-like malignant melanomas (SLMM), the latter tumors having initially been interpreted as various types of soft tissue sarcoma, such as fibrosarcoma, neurofibrosarcoma, malignant fibrous histiocytoma and liposarcoma, were studied. All the CCS were positive for S-100 protein and, ultrastructurally, features of both Schwann cell and melanocytic differentiation were observed. Eight/15 patients died of metastatic tumor disease. Seven/14 EMS were positive for S-100 protein and the electronmicroscopic findings indicated a Schwann cell differentiation. Eleven/14 patients died of tumor disease. The 29 CS were characterized light microscopically by high cellularity and a spindle cell and fascicular appearance and a certain mitotic activity. All the cases followed a benign clinical course. Some of the tumors had been erroneously diagnosed as soft tissue sarcoma. Ultrastructurally a high degree of Schwann cell differentiation was found and immunohistochemically 27/29 and 29/29 were positive for S-100 protein and glial fibrillary acidic protein (GFAP) respectively. A cell culture and cytogenetic analysis performed in one case of CS revealed a diploid stemline with a variety of abnormal clones, including one with monosomy for chromosome 22. A correlated radiographic and histopathologic study was performed on 17 cases of peripheral schwannoma (neurilemoma) (PS). At plain radiography the tumors were found to be surrounded by a radiolucent rim corresponding to adipose tissue. At angiography 6/8 were hypervascular and in three cases hypertrophied nutrient nerve vessels were observed, and at un-enhanced computed tomography (CT) 14/16 were homogeneous, while 15/16 showed inhomogeneously increased attenuation following contrast enhancement. The SLMM showed melanosomes ultrastructurally in 13/16 tumors and a positivity using anti-S-100 protein antibodies and the anti-melanoma antibody NKI/C3 (29/29) and anti-melanoma antibody HMB 45 (9/21) was noted immunohistochemically. The majority of the tumors occurred adjacent to lymph node stations, most commonly the groin and axilla. A primary cutaneous malignant melanoma could be demonstrated in 11/21 patients following a review of their history and renewed physical examinations.

Conclusions: 1) CCS is a homogeneous entity showing both melanocytic and Schwann cell differentiation, thereby supporting a neural crest derivation. 2) EMS are highly malignant tumors with morphologic and immunohistochemical properties indicating a Schwann cell differentiation. 3) PS has a characteristic radiographic appearance, which can contribute to a preoperative diagnosis. The inhomogeneous CT appearance of PN can be related to the distribution of vessels in the tumors. 4) CS is a variant of schwannoma with a benign clinical course, and it is suggested that it should be included in the group of pseudosarcomas. CS is characterized by a high degree of Schwann cell differentiation immunohistochemically and ultrastructurally. The consistent expression of GFAP in CS suggests that it may originate from a population of non-myelin forming Schwann cells. 5) Cutaneous malignant melanomas of classical appearance can give rise to metastases which clinically and light microscopically closely resemble various types of soft tissue sarcoma. Electronmicroscopy

for the demonstration of melanosomes and immunohistochemistry for the demonstration of S-100 protein and melanoma-associated antigens are important tools for the recognition of these tumors.

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Ultrasound in the diagnosis and treatment of ovarian tumours

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Ultrasound has been increasingly used during the the last few years for evaluation of tumours in the lower pelvis of women of all ages. Most of our knowledge of the ultrasound image of such tumours is based on abdominal ultrasound scanning. With the introduction of high-resolution endovaginal ultrasound, a new diagnostic tool has become available for studying ovarian tumours.

Simple cystic ovarian tumours are common in menstruating women and not seldom found in postmenopausal women. One might then ask what are the chances of a simple ovarian cyst's being malignant and is endovaginal ultrasound an accurate method of characterising such a tumour? It is well known that laparotomy carries a high risk of infertility problems in menstruating women and a higher risk of postoperative complications in older women. Ultrasound-guided puncture of cystic ovarian tumours could thus be an alternative to laparoscopic puncture or surgery.

By ultrasound, it was possible to identify and measure 87% of the postmenopausal ovaries, as compared to 30% identified at the ordinary gynaecological examination. Volumes less than 1 cm^3 could not be palpated. In a retrospective study of 1 017 women operated upon due to pelvic tumours, 296 tumours were classified as unilocular (simple cyst) on macroscopic characterisation. Only one was malignant. This tumour had macroscopically visible vegetation inside the cyst-wall. In two prospective studies of 230 pelvic tumours, endovaginal ultrasound revealed 65 unilocular cystic ovarian tumours and classified these as being benign, which agreed with the histopathological diagnosis. The specificity and sensitivity of endovaginal ultrasound for classifying a pelvic tumour of being malignant were 90% and 87% respectively. The corresponding figures found at the cytological evaluation of the cyst content of a punctured cystic tumour were 100% and 47% respectively. In 96% the endovaginal ultrasound image agreed with the macroscopic characterisation of the tumour.

Ultrasound-guided puncture of symptom-giving cystic ovarian tumours resulted in cure in 70% of the women, indicating that the method is a good alternative to surgery. Fifty-one per cent of those women who were punctured under endovaginal ultrasound guidance did not need any anaesthesia at all.

Endovaginal ultrasound seems to be a reliable method for characterisation of pelvic tumours. Simple ovarian cysts seem to carry a very low risk of malignancy and can be diagnosed very accurately by endovaginal ultrasound. Puncture of such a cyst under endovaginal ultrasound guidance is a safe and good alternative to surgery.

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Management and prognosis of patients with high-grade soft tissue sarcomas—An evaluation of a Scandinavian joint care program

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Doxorubicin is one of the most active single agents in metastatic soft tissue sarcoma. This was the rationale for the evaluation of doxorubicin in an adjuvant situation for patients with high-grade soft tissue sarcoma in a randomized, Scandinavian, multicenter joint care program, which was carried out from January 1981 to February 1986, and included 240 patients. These patients comprised the base series for the investigations of the importance of the surgical margin and radiotherapy for local tumor control; the accuracy of malignancy grading and tumor typing; prognostic factors, including DNA content; and epidemiologic risk factors.

The results showed that the use of doxorubicin as adjuvant chemotherapy did not have clinical benefit in patients with high-grade soft tissue sarcoma.

The overall local tumor control was high (94%) for radically operated on extremity-located tumors due to strict classification of surgical margins. The only risk factor for local recurrence was marginal surgery without radiotherapy with a four times higher risk than after compartmental or wide surgery. Twelve percent of the operations reported as radical were reclassified as marginal, demonstrating the importance of reevaluation of surgical margins, and in these patients the local recurrence rate was 37%. One-fourth of the tumours were reclassified as regards tumor type and 40% as regards malignancy grade. Reclassification of 20 tumors resulted in ineligibility for the trial, underlining the importance of histiologic review in sarcoma studies. Also grading highly malignant soft tissue sarcoma in two grades (grades III and IV) increased the prognostic information.

Histologic malignancy grade IV, increasing tumor size, vascular invasion by the tumor, male sex, and DNA aneuploidy were identified as independent prognostic factors. The 5-year metastasis-free survival for the whole patient series was 55%, with the extremes 79% for the patients with no or only one risk factor and 0% for those with four or five risk factors. The five prognostic factors found could be used for selection of high-risk patients for adjuvant chemotherapy in the future.

The epidemiologic study gave limited support for an association between occupational phenoxy-acid exposure and soft tissue sarcoma development.

The work described in this thesis was performed at the Lund University Hospital, Departments of Oncology, Pathology, Clinical Cytology, and Orthopedics, in close collaboration with the Scandinavian Sarcoma Group.

December 1989

Role of immunosuppression in radiostrontium-induced oncogenesis—A histopathological and immunological study in ^{90}Sr-exposed normal and immunocompromized CBA mice

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The tumourigenic and immunosuppressive effects of ionizing radiation from incorporated ^{90}Sr and its daughter nuclide ^{90}Y and the possible relationship between tumour response and immune failure were studied experimentally in mice.

Young adult, male CBA/SU mice were injected with various amounts of ^{90}Sr (optimal and suboptimal for osteosarcoma induction), and in addition, subgroups were immunosuppressed by adult thymectomy (ATx) and/or prolonged antilymphocyteglobulin (ALG) treatment.

All mice were autopsied after spontaneous death and examined histologically for neoplastic lesions, and crude and actuarial tumour values were calculated. The immunological competence of the mice was estimated in terms of (a) lymphocyte responsiveness to mitogens, (b) reticuloendothelial phagocytosis, (c) natural killer

(NK) cell lytic activity, (d) peripheral blood T-cell numbers, and (e) allogenic skin grafting. An evaluation of the tumour responses (mainly osteosarcomas and malignant lymphomas), including incidence, latency, histologic type, location, multiplicity, progression and metastasis, revealed that differences between mouse groups were exclusively related to the amount of ^{90}Sr injected. It was therefore concluded that severe immuno-deficiency, as induced by ATx and ALG treatment, did not, at any dose-level of ^{90}Sr, render the animals more prone to develop tumours, nor did it influence tumour formation in any other way. The fact that no decisive or modulating influence of the immune system on the development of ^{90}Sr induced tumours could be found suggests that these tumours are either non-immunogenic or very weakly immunogenic, or possibly capable of evading immune rejection. However, the implications of ^{90}Sr induced NK-cell suppression should be further investigated before immuno-deficiency can be ruled out as an etiologic cofactor in ^{90}Sr oncogenesis. Preneoplastic proliferation of bone marrow stromal cells (dysplasia and nuclear atypia) preceded the development of osteosarcomas, as evidenced by their corresponding locations and incidences. The histopathology of these lesions strongly suggests that many osteosarcomas originate from undifferentiated stromal cells and may, later during progression, express various bone tumour characteristics and histologic patterns.

December 1989

Cellular heterogeneity in endocrine tumours—A morphological and immunohistochemical study of medullary carcinoma of the thyroid and pituitary adenomas

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Both pituitary ademonas and medullary carcinoma of the thyroid express multihormonality. Six hormones were observed in the null cell adenomas of the pituitary gland. Three of them, viz. gastrin, β-endorphin and CGRP, have to our knowledge not previously been found in such tumours. Great immunohistological heterogeneity was found in medullary carcinomas and as many as seven different hormones and serotonin were identified within the same tumour.

Ultrastructural and immunoelectron microscopic examinations showed that the pituitary adenomas producing both GH and PRL were rather complex tumours, and as many as five different cell types were observed within the same tumour. These cells produce either GH or PRL or both. Our study clearly showed that immunoelectron microscopy with single and double staining methods was necessary to distinguish cells having the morphology of 'pure' GH cells, but containing either GH or PRL, or both.

In medullary carcinoma of the thyroid large variations in size and density of secretory granules were found. Cells with small granules, medium sized and large granules were observed within the same tumour. The medium and large sized granules were either dense-cored or pale-cored. The previous separation into type I and type II granules is an oversimplification. Immunoreactivity for calcitonin and CGRP was identified in all different granule types.

The detection of immunoreactivity for thyroglobulin and calcitonin, or thyroglobulin and CGRP in the same neoplastic cells in both primary tumours and in a lymph node metastasis supports the existence of true MCT coexpressing thyroglobulin. These findings also exclude the hypothesis that thyroglobulin-positive MCT are derived from both C-cells and follicular cells. The histological features were similar in thyroglobulin-positive and thyroglobulin-negative MCT, but the prevalence of somatostatin-positive cells was significantly greater in tumours that express thyroglobulin than in those that did not.

In medullary carcinoma of the thyroid the production of multiple peptide hormones or amine or both carries no prognostic significance.

In our study patients with thyroglobulin-positive MCT had a longer survival than individuals with thyroglobulin-negative MCT, but the number of cases was too small to allow generalisations.

January 1990

Karyometry and DNA-cytophotometry of naevi and cutaneous melanomas—A study comparing naevi and melanomas and referring to prognosis of melanomas

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Data concerning the nuclei of pigmented cell tumours is rather scanty in medical literature and for that reason karyometry and single cell DNA-cytophotometry was done.

In a karyometry study it was found that the nuclear size of benign compound naevi differed from that of Spitz naevi and melanomas.

In a single cell DNA-cytophotometry study it was further found that the differences in nuclear optical density differentiated Spitz naevi from melanomas and common, acquired naevi. Quantitative data on melanoma nuclei was obtained by karyometry, single cell DNA-cytophotometry and flow cytometry and was related to prognosis.

A material consisting of 82 cutaneous clinical stage I melanomas from 1972 to 1974 was examined using karyometry; and the results were related to clinical, histopathological and prognostic data. By using Cox's multivariate analysis it was found that variability of mean nuclear area (expressed as percentile 90 minus percentile 50 of mean nuclear area), standard deviation of form factor and mean maximal nuclear diameter added prognostic information even if melanoma thickness or Clark level of invasion were introduced into the analysis.

Using single cell DNA-cytophotometry on 50 of the above melanomas, no influence of ploidy on prognosis could be found—while karyometrical findings obtained continued to be related to the prognosis.

A material consisting of 82 paraffin-embedded primary melanomas from 1978 to 1981 was examined with flow cytometry. A single parameter test demonstrated that diploid melanomas had better prognosis than aneuploid ones. Nevertheless, a multivariate analysis demonstrated the influence of only melanoma thickness or Clark level of invasion.

February 1990

A population-based survey of neoplasms of the central nervous system in Norway

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The data on CNS neoplasms in the Norwegian Cancer Registry is sufficiently valid for epidemiological analysis of the total group and histological groups.

The case ascertainment of primary intracranial neoplasms declines after 60 years of age. High autopsy rates and availability to computerized tomography (CT) improve the ascertainment in the elderly.

Below 60 years of age a stable trend in glioma incidence is observed through three decades of cancer registration.

For glioma patients survival prospects relate to histological type and age at the time of diagnosis. The most favourable prognosis is seen for astrocytoma patients below 30 years and the least favourable is seen for glioblastoma patients. For oligondendrogliomas, however, the survival prospects are independent of the age of the patients.

In order to collect an adequate number of glioma patients for epidemiological analysis according to histology it is appropriate to analyse glioblastoma and medulloblastoma as separate entities and the remaining glioma types as a composite group ('other gliomas').

The female predominance in meningioma incidence is ascribed to the middle-age groups. This finding may imply a rôle of female sex hormones in the development of meningiomas. A significantly raised incidence of meningiomas in birth-cohorts after 1930 should be followed more closely during the next decade with a view to evaluating the possible effect of anti-conception pills.

The Norwegian data show a significant association between breast cancer and meningioma. Female sex hormones are, at present, the most plausible explanation for the association. Male meningioma patients have a statistically increased risk of developing renal cancer.

The time trend for primary intraspinal neoplasms is stable over the period 1955–1986 indicating an unaltered diagnostic sensitivity. Glioblastoma is a very rare tumour type intraspinally, contrary to the situation intracranially. Patients with intraspinal meningiomas or ependymomas have a significantly better 5-year relative survival than those with intracranial tumours.

The pre-morbid height is a significant risk factor for later development of CNS neoplasm particularly for male glioblastoma patients and for female patients with 'other gliomas'. Quetelet's index (weight/height squared) emerged negatively correlated with later occurence of 'other gliomas' in females. For meningioma patients no significant association was revealed with Quetelet's index.

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Carcinoembryonic antigen (CEA)—Tumor specificity of monoclonal antibodies to CEA and molecular cloning of cDNAs for CEA-related salivary gland antigens

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Carcinoembryonic antigen, CEA, is a 180 kD glycoprotein that is used extensively as a tumor marker in colorectal and other carcinomas. CEA belongs to a family of cross-reactive antigens most of which occur in normal tissues. This cross-reactivity makes the CEA-assays inaccurate. In the CEA-gene family 17 genes are known, so far. These have been divided into two subgroups: The CEA/NCA subfamily and the pregnancy specific glycoprotein (PSG) subfamily. The CEA-gene family belongs to the immunoglubulin gene super family.

The tumor specificity and cross-reactivity of a group of monoclonal antibodies (Mab) against CEA was studied. Twelve Mabs were characterized by reactivity in ELISA, using purified CEA and CEA-related antigens, and by immunohistochemistry, using a large panel of normal or tumorous human tissue. Three Mabs were found to be highly CEA-specific and could, therefore, be selected as potentially good reagent for clinical use.

Two colon carcinoma cell lines (LS174T and HT-29) were studied for expression of CEA and MHC antigens after treatment with IFN-γ, sodium butyrate or PMA. IFN-γ, enhanced CEA expression 2-fold in LS174T cells and 6–8-fold in HT-29 cells. Even higher induction was observed when these cell lines were stimulated with sodium butyrate or PMA. Furthermore, IFN-enhanced MHC class I antigen expression in both cell lines, while the MHC class II antigens were only induced in the HT-29 cells.

Human submandibular salivary gland and saliva were found to contain a series of CEA-family glycoproteins, using immunohistochemistry, immunoprecipitation and SDS-PAGE electrophoresis. Three major glycoproteins were identified with an apparent molecular weight of 85, 65 and 31 kD. In Northern blot analysis, at least 8 different mRNA species were found, the quantitatively dominating species coding for NCA 55/95. Three mRNAs coded for different spliced forms of BGP, at least 2 coded for different members of the PSG subgroup and 1, perhaps 2, coded for unknown CEA-family members.

Three CEA-family cDNA clones were isolated from human submandibular salivary gland. Sequence analysis of these 2.0, 1.7 and 0.7 kB clones showed that they coded for three different but closely related PSG molecules.

December 1989

Recessive mutations in tumorigenesis of human breast carcinoma and multiple endocrine neoplasia type 1

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Initiation of tumorigenesis in heritable tumors have been suggested to occur because of mutations unmasking recessive mutations. These predictions have been confirmed in the past few years by molecular studies of the eye tumor retinoblastoma. To study whether this is a general mechanism for tumorigenesis, two other types of heritable tumors were investigated.

Multiple endocrine neoplasia type 1 (MEN1) is a predisposition to hyperplasia or tumors of the parathyroid glands, anterior pituitary and the endocrine pancreas, which is inherited as an autosmal dominant trait. A locus for the disease was identified by different methods. First, specific chromosomal rearrangements of chromosome 11 were identified in two insulinomas from two brothers who had inherited the disease from their mother. Determination of the parental origin of the lost chromosomes showed that the tumors occurred after elimination of the normal chromosome, suggesting that the MEN gene belongs to the group of recessive cancer genes. Subsequent linkage analysis in families confirmed the putative localization.

As a first step towards identification of the gene, the region of interest was restricted to a few million base pairs by construction of a genetic linkage map around the MEN1 locus and deletion mapping in tumors. The immediate clinical application of these findings is that accurate presymptomatic diagnosis can be performed by molecular genetic methods.

Human breast cancer is a heterogenous genetic disease in the sense that it is a major component of several heritable syndromes. Chromosomal rearrangements detected as loss of constitutional heterozygosity was identified on several chromosomes. Allele losses affecting different chromosomes could be associated with subsets of tumors. For example, chromosome 13 in premenopausal carcinomas, chromosome 17 in ductal carcinomas

and chromosome 22 in lobular carcinomas. Furthermore, patients who developed an advanced stage of the disease after many years of mild clinical course showed a higher frequency of allele losses in their primary tumors compared to cases with a continuously mild disease.

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Autopsy studies of the occurrences of cancerous, atypical and benign epithelial lesions in the female breast

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The main purpose of the present thesis has been to estimate the frequency of malignant, premalignant and benign morphological changes in the female breast and evaluate their possible interrelationship. The method used was a conventional but detailed and systematic examination, both macroscopically and histologically of all available breast tissue. Consequently autopsi material has been the natural basis of the studies.

Three groups of women have been examined:

a). A consecutive series of 110 younger women, mean age 39 years, undergoing medicolegal autopsy at the Institute of Forensic Medicine in Copenhagen, Denmark, from October 1983 to July 1984.

b). A consectutive series of 83 unselected, elderly women, mean age 67 years, undergoing autopsy at a large community hospital in Esbjerg, Jutland, Denmark, from November 1976 to May 1977.

c). A consecutive series of 84 women, mean age 74 years, dying with a known diagnosis of breast cancer and undergoing autopsy at two community hospitals in the Copenhagen area, (Glostrup and Frederiksberg), Denmark, from November 1982 to December 1984.

The main results were:

1. In younger women clinically occult in situ breast cancer lesions occurred with surprisingly high frequency, 18%, a result which may influence the planning of future screening programs.
2. In elderly women, dying from a number of various reasons, invasive breast carcinomas were found with a frequency corresponding to the life-long risk of Danish women having this disease diagnosed clinically, 8%. Occult in situ carcinomas were found in 18%.
3. In women dying with a clinical diagnosis of breast cancer, the frequency of malignant histological changes in the opposite breast (metastases, invasive and in situ breast carcinomas) was unexpectedly high, 80%, a result which should be taken into consideration during clinical follow-up after treatment for breast cancer.

1989

Studies of chemically-induced tumour promotion using the altered hepatic foci bioassay

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Chemically-induced carcinogenesis in laboratory animals may be visualized as a multistep process involving definable stages termed initiation, promotion and progression. Several non-genotoxic, halogen-containing environmental pollutants may act as experimental carcinogens, apparently by effecting the promotion step without significantly altering the other stages. The process of tumour promotion is considered to be reversible, as opposed to initiation and progression. Therefore, the characterization of an agent as a tumour promoter without genotoxic qualities may have considerable bearing on the final assessment of the risk of human cancer from exposure to such an agent.

The objectives of the present study were to investigate tumour promotion-related effects induced by some organohalogen compounds using in vitro and in vivo approaches. A number of pesticides were used as model substances in studies on promoter-induced inhibition of intercellular communication (IC) in vitro and enhancement of altered hepatic foci (AFH) development in carcinogen-initiated rats in vivo. Furthermore, modulation of TCDD- and phenobarbital-induced promotion of AHF-development by vitamin A supplementation and the type of diet (purified, casein-based diet vs conventional, cereal-based diet) was investigated.

A reasonable correlation was found between the results of the assays of chemically-induced inhibition of IC in vitro and enhancement of AHF-development in rat liver among some structurally/functionally related DDT-analogs and pyrethroids. The ostensible relationship between liver tumour promoting activity and induction of liver growth/enzyme activities was examined in an initiation/promotion-experiment. Among DDT and four DDT-analogs all but one (fenarimol) acted as tumour promoters and enhanced the development of GGT-positive AHF in nitrosamine-initiated male rats. However, all of the DDT analogs studied, including fenarimol, were found to be potent inducers of liver growth and of cytochrome P450b-related isoenzyme activities. Thus, the present results do not support the suggestion of a strict correlation between liver growth/PB-type enzyme induction and liver tumour promoting activity.

In the study of modification of TCDD- and phenobarbital (PB) induced enhancement of nitrosamine-initiated AHF-development by dietary vitamin A supplementation and the type of diet, vitamin A deficiency was shown to potentiate the effect of TCDD. The effect of PB was more or less unaffected by varying vitamin A supplementation. Furthermore, severe vitamin A deficiency alone enhanced the development of AHF. Thus, the present results further emphasize the importance of adequate levels of this vitamin in the diet. Under conditions of vitamin A deficiency, TCDD was shown to induce oval cell hyperplasia, a lesion that has been suggested to be a precursor of liver cancer. A differential effect on AHF-development by the two types of diet was apparent. From this study it was concluded that TCDD-induced depletion of hepatic stores of vitamin A is not a primary mechanism in liver tumour promotion induced by this compound. However, it may well be that TCDD-induced vitamin A deficiency acts as a tumour promoting stimulus concertedly with an, as yet, unidentified mechanism of tumour promotion by TCDD.

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Epidemiological studies on urothelial cancer

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The purpose of this study programme was to identify risk factors for urothelial cancer. Three study bases were depicted; two cohorts were followed up and a case–referent study was made in the county of Stockholm in 1985–1987.

Aromatic amines, cigarette smoking and phanacetin are some factors that previously have been linked to urothelial cancer. In

this study, male pipe smokers had about a 3-fold increase in the risk of urothelial cancer as compared to men who have never smoked.

Intake of supplements mainly containing vitamin A was associated with a reduction in the risk of urothelial cancer. Although based on small numbers, the results indicate a stronger effect with increasing frequency of consumption. No association was found, however, with dietary vitamins.

In a cohort study, subjects with a high intake of beef and pork had an increased risk of urothelial cancer, and we postulated that intake of browned material formed during cooking is important for the occurrence of urothelial cancer. This hypothesis was supported in a case–referent study—several fried foods were associated with urothelial cancer. A dose–response relationship was also seen with an increasing average daily intake of fat, but there is some concern considering a residual study base error. No increase in risk was seen for meat other than fried, but the analysis was based on small numbers.

A cohort study gave some support for combustion gases from coal, creosote and polychlorinated biphenyls (PCB) being associated with urothelial cancer and warranted further attention to chlorinated aliphatic hydrocarbons and cutting fluids and cutting oils. In a case–referent study we found an increased risk after exposure to benzene. Although based on small numbers, the results indicate that the highest annual dose gives the highest risk, and that the exposure has to start more than two decades before the observation period if it is to have an effect. An increased relative risk after exposure to exhausts diminished after adjustment for benzene. In an evaluation of the epidemiological literature we conclude that among the industry-related chemicals only certain combustion gases from coal, besides aromatic amines, have convincingly been linked to urothelial cancer.

March 1990

Effects of selenite-induced variation in glutathione peroxidase activity on cellular resistance to radiation- and peroxide-induced free radical damage

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The activity of selenium-dependent glutathione peroxidase (glutathione:hydrogen peroxide oxidoreductase, EC 1.11.1.9) was manipulated in nine mammalian cell lines by means of variation in culture medium contents of selenite and serum. Selenite induced in most of the cell lines an increase in glutathione peroxidase activity, but the responses varied considerably and the selenite-induced increases ranged from negligible to more than ten-fold. Three cell lines, the human colon carcinoma HT29, the human mesothelioma P31, and the mouse neuroblastoma N-18, were used to investigate the effects of variable glutathione peroxidase activities on radiation resistance. The activities of the other intracellular antioxidant enzymes, CuZn superoxide dismutase, Mn superoxide dismutase, catalase and glutathione transferases varied only marginally after selenite supplementation. Control and selenite-supplemented cells were exposed to different doses of gamma radiation. There were no changes in the surviving fractions. Neither were there any significant differences between the two groups in the induction of DNA strand breaks after gamma-irradiation under repairing (37°C) or non-repairing (0°C) conditions.

The effects of the obtained variation in glutathione peroxidase activities were also investigated by exposing HT29 and P31 cells to hydrogen peroxide and tert-butyl hydroperoxide. Selenite supplementation resulted in a decrease in hydrogen peroxide-induced DNA single-strand breaks in both HT29 and P31 cells. A small, but significant, reduction in the number of DNA single-strand breaks for low doses (10–50 μmol/l) of tert-butyl hydroperoxide was only found in P31 cells. In spite of the protective effect of increased glutathione peroxidase activity on DNA single-strand break formation there were no apparent differences between selenite-supplemented and unsupplemented cells in cell survival after peroxide exposure. The results suggest that selenium-dependent glutathione peroxidase does not significantly contribute to the radiation resistance of cultured mammalian cells and that hydroxyl radicals generated from hydrogen peroxide are relatively unimportant in ionizing radiation-induced cell killing.

May 1990

Chronic myeloproliferative disease with thrombocytosis—A clinical, morphological and cytogenetic study with emphasis on effects of hydroxyurea therapy

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Thrombocytosis caused by a malignant haematopoietic cell clone with increased autonomous platelet production is a common finding in chronic myeloproliferative disease. Symptoms, complications, and effects of treatment in 81 patients with thrombocytosis and polycythaemia vera (PV), essential thrombocythaemia (ET), or myelofibrosis (MF) are discussed in the present work. The study also included pretreatment platelet aggregation studies in 19 of the patients. The aggregation studies revealed a uniform pattern with raised ADP threshold, abolished second wave adrenalin aggregation and impaired ATP secretion.

It is known that myelosuppressive therapy such as irradiation and alkylating drugs is leukaemogenic, necessitating trials of other agents for control of thrombocytosis, splenomegaly, and other symptoms and complications not controlled by phlebotomy. This study presents results of long-term, continuous treatment of patients with PV, ET and MF with hydroxyurea, and S-phase specific drug inhibiting ribonucleotide reductase. Hydroxyurea induced macro-megaloblastic blood and bone marrow changes. Rapid control of the platelet count was achieved. Control of disease-related symptoms was obtained in 80% of the patients during the 1st year, and toxicity of hydroxyurea was low. 27% of the patients failed on hydroxyurea therapy. Crude survival was excellent with 86% probability of survival after 5 years.

Bone marrow fibrosis is common in the chronic myeloproliferative disorders. This process is thought to be mediated by platelet derived growth factor (PDGF), released from megakaryocytes. Testing the hypothesis that suppression of megakaryocyte proliferation would diminish the risk of bone marrow fibrosis, we aimed at normal platelet counts during hydroxyurea treatment. Data are presented that support the conclusion that treatment resulted in reversal of reticulin fibrosis by suppression of megakaryocyte proliferation. Myelodysplastic syndrome and acute leukaemia, a known complication in these disorders, developed in 4/81 patients on hydroxyurea. The frequency of cytogenetic abnormalities was low.

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Clinical pharmacological aspects of 6-mercaptopurine in maintenance therapy of childhood leukemia

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Following remission, children with acute lymphoblastic leukemia receive a long-term oral remission maintenance therapy with 6-mercaptopurine (6-MP) daily and methotrexate (MTX) weekly, administered according to a standardized protocol. In spite of this, 40% of the children relapse. One cause for relapse may be ineffective maintenance therapy due to a low and variable bioavailability of 6-MP.

In this thesis the pharmacokinetics of 6-MP have been evaluated and related to intake of food, to the concomitant methotrexate (MTX) treatment and to the clinical outcome in 22 children with acute lymphoblastic leukemia.

The study gave the following results:

1. The administration of the oral long-term remission maintenance therapy with 6-MP in acute lymphoblastic leukemia leads to unpredictable plasma concentrations with pronounced inter- and intra-individual variation.
2. The pharmacokinetics of 6-MP is not significantly influenced whether or not 6-MP is administered on an empty stomach or after food intake.
3. Dose intake together with MTX does not significantly alter the pharmacokinetics of 6-MP.
4. The clinical outcome was correlated to the mean plasma concentrations during the treatment. Five patients with a mean area under the concentration versus time curve, measured during 4 hours, (AUC_{0-4h}) of <270 ng/ml × h (<1.75 μmol/l × h) relapsed during maintenance therapy, while 4 patients with mean $AUC_{0-4h} > 370$ ng/ml × h (>2.42 μmol/l × h) developed severe toxicity.
5. Prediction of plasma AUC_{0-4h} for 6-MP is possible in the individual patient by means of a simplified sampling schedule.
6. The pronounced inter-individual variation in plasma 6-MP concentrations indicates the need for individualizing the dose by therapeutic drug monitoring, instead of using a standardized dosing protocol. The intra-individual variation in plasma 6-MP concentrations indicates the need for repeated analysis. Such measurements would make it possible to individualize the therapy and reduce the relapse risk in the remission maintenance treatment.
7. Studies in murine leukemia cells in vitro indicate a paradoxical dose–effect response with reduced cytotoxicity at incubation concentrations of 6-MP above 1–2 μmol/l. Most patients do not reach this peak plasma concentration.

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The carcinoembryonic antigen gene family—Molecular analysis of the pregnancy-specific β1 glycoprotein subgroup

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Carcinoembryonic antigen (CEA) is a tumor-associated antigen expressed in carcinomas of the gastro-intestinal tract particularly in the colon/rectum. CEA is widely used as a tumor marker and is considered to be an oncofetal antigen. To investigate fetal forms of CEA we screened a first trimester human fetal liver cDNA library using synthetic DNA probes for CEA. A new family of CEA-related antigens, termed fetal liver non-specific cross-reactive antigen (FL-NCA), was identified. The FL-NCAs proved to be identical to pregnancy-specific β1 glycoproteins (PSG) recently isolated and sequenced from an human placental cDNA library. PSG are associated with pregnancy and have been known at the biochemical level for twenty years. Four different FL-NCAs were sequenced and expressed. They are now termed PSG1d, PSG1a, PSG5 and PSG11 (nomenclature, Tumor Biol 1990; 11: 5–19). PSG1d, PSG5 and PSG11 are new. The PSGs constitute a subgroup within the CEA gene family which, in its turn, belongs to the immunoglobulin gene superfamily. All PSGs are made up of a single N-terminal domain (N) homologous to the variable region of immunoglobulin, varying numbers of internal domains (A, B) and a short C-terminal domain. The A- and B-domains correspond to the constant region of immunoglobulin (C2 type). PSG1 and PSG11 can be written as N-A1-A2-B2-C and PSG5 as N-A2-B2-C. The latter domain arrangement is unique. The PSGs are secreted glycoproteins with molecular weights of 54 to 72 kD.

Seventeen genes have so far been identified in the CEA gene family. Of these, 6 belong to the CEA-NCA subgroup and 11 to the PSG subgroup. The size of the PSG subfamily was estimated as 13 or a multiple thereof. The PSGs are very similar to each other (70–97% homology at the amino acid level if individual domains are compared). Interestingly, two regions in the N-domain show sequence variability when different PSGs are compared with each other. These regions correspond to complementarity determining regions 2 and 3 of immunoglobulin (CDR-2 and 3). The variability in the CDR-3 region is most prominent. This region also contains the RGD sequence in 5 of the 9 PSGs whose sequence is known. The PSGs may be involved in cell/cell, cell/matrix interactions utilizing the RGD sequence in the N-domain.

Human submandibular salivary gland and saliva contained several CEA-related antigens. Immunochemical analysis revealed the presence of 5 glycoproteins (molecular weights: 85, 65 (major component), 50, 35 and 30). The 85, 65 and 30 kD components were produced and secreted by the mucous secreting cells into the saliva. mRNA analysis showed that the gland contained the following CEA-related antigens: NCA 55/95 mRNA, biliary glycoprotein (BGP) splice variants a and c mRNA, 2–3 PSG mRNAs and possibly one unknown mRNA species (1.6 kB). The major 65 kD glycoprotein is most likely the product of the NCA 55/95 gene. The size indicates tissue specific glycosylation. Analysis of a cDNA library from submandibular salivary gland demonstrated the presence of PSGs. PSG1d, PSG2 and PSG3 were fully identified by sequencing. This study shows conclusively that the so-called pregnancy specific glycoproteins can be expressed in normal adult tissues other than placenta.

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