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.

Selection of putative preneoplastic hepatocytes

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Carcinogen-induced alterations in enzyme phenotype may confer resistance to preneoplastic cells during hepatocarcinogenesis. Selection of resistant hepatocytes is suggested to be one possible mechanism for clonal expansion of preneoplastic cells. The mechanisms leading to such selection are not completely understood. The aim of the present study was to investigate mechanisms of selective resistance that may lead to clonal expansion of foci with an altered enzyme composition (EaF).

An in vitro model for studies of resistance with normal and preneoplastic cells was developed. Isolated hepatocytes from carcinogen-treated rats, with a high yield of EaF, were used and effects on cell attachment were studied as a critical effect.

Gamma-glutamyltranspeptidase (GGT) is a marker enzyme for EaF and has been suggested to provide resistance against toxicity induced by depletion of intracellular glutathione (GSH). We found that GGT-positive cells are resistant to toxicity induced by GSH depletion and oxidative stress and that this resistance may be related to the GGT activity. Collagen attachment receptor (β_1 integrin) was found to be a target molecule and was selectively altered on GGT-negative cells.

Phorbol ester-induced resistance was found to be another factor that protected cells against decreased attachment. Decreased cell attachment induced by GSH depletion and TPA-induced resistance in GGT-positive cells were two critical factors in phorbol ester-induced selection.

GSH depletion was found to be a factor that may lead to the selective growth of foci in vivo. We also found a correlation between the capacity to induce growth of EaF in vivo and to induce selection in vitro for a group of benzene derivatives, indicating the in vivo relevance of our in vitro model.

In conclusion, this study provides evidence that resistance in putative preneoplastic hepatocytes may involve altered cell matrix receptor function in response to GSH depletion and oxidative stress and that this resistance may lead to clonal expansion of EaF.

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Prediction of prognosis in human breast cancer—A study on clinicopathologic and cytometric prognostic factors

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This study was undertaken to evaluate some important prognostic factors in human breast cancer. The prognostic value of accepted clinicopathological factors such as the presence of axillary lymph node metastases, histologic grade, clinical and pathological stage was confirmed.

In a cohort of stage T3, T4, M0 breast cancer with 91 patients (paper I) DNA ploidy by static cytometry (SCM) turned out to be the most important prognostic factor. In a cohort of stage T2, M0 breast cancer with 99 patients (paper III) the presence of involved axillary nodes and low histologic grade were independent prognostic factors. According to life-table analyses DNA ploidy by flow cytometry (FCM) and SCM were significant prognostic predictors for survival but S-phase fraction (SPF) was not. The significant discrimination between euploid and aneuploid tumours was seen also among the node-negative patients. In a patient material with 158 tumours of predominantly low stages (73% T0, T1, papers IV and V) and calculated mammographic tumour volume doubling time (DT) DNA ploidy by FCM gave no significant prognostic information. A computer program was used to calculate SPF from the histograms obtained by FCM. SPF with a cut-off value of 7.5% between tumours with high and low proliferation rate was a highly significant and independent prognostic factor for survival. The other independent prognostic predictors were low histologic grade, the presence of involved axillary nodes and stage II and III (versus stage I).

DT values for 158 patients (paper IV and V) varied between 0.6 and 65.8 months (mean 10.9 months) and 11 tumours showed no growth at all between mammographies. The median value of 9.0 months was chosen as cut-off point between slow and fast growing tumours. The prognostic power of DT was, however, low, and the difference between slow and fast growing tumours was significant only for distant disease-free survival. Seventy-one of the 158 tumours were detected by mammographic screening. The screening detected carcinomas with predominantly long DTs were discovered at an early stage and showed favourable characteristics concerning DNA ploidy and SPF.

FCM was a rapid and reliable method for DNA analysis with a better prognostic discrimination between euploid and aneuploid groups than SCM (papers II and III).

SPF, DNA ploidy and histologic grade are significantly correlated to one another but show no strong correlation to the presence of axillary lymph node metastases. There is also a significant correlation between DT on one hand and DNA ploidy and SPF on the other hand.

In conclusion the classic prognostic factors are still valuable. DNA ploidy as a single prognostic factor seems to have a relatively low prognostic power and seems to be of limited clinical value. SPF is a highly significant prognostic predictor for breast cancer of low stage, but the clinical value is not defined.

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The role of the glucocorticoid receptor and chromatin structure in the induction of the MMTV promoter

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Transcription from the promoter in the long terminal repeat of the mouse mammary tumour virus (MMTV) is activated by glucocorticoids. This hormone acts by binding to the glucocorticoid receptor (GR) which interacts in a hormone-dependent manner with specific sequences within the regulatory region of the MMTV promoter. A possible mechanism for promoter activation has been suggested by the observations that a specifically positioned nucleosome in the promoter is disrupted as a consequence of hormone treatment. This results in increased

accessibility of the DNA and may 'open' the promoter for additional factors.

The present work involves the analysis of GR-interaction with protein-free as well as nucleosome organized MMTV DNA. In the interaction with the MMTV regulatory region, GR binds with high affinity to two binding regions of functional importance. Although GR bound to these regions synergizes to activate transcription, the basis for this synergism appears not to be cooperative DNA binding. Also the synergism between GR and NF-1, another transcription factor interacting with the promoter, could not be explained by cooperative DNA binding.

To evaluate the GR interaction with MMTV DNA folded into a nucleosome, a cloned MMTV fragment was used for to reconstitute nucleosomes in vitro. Even when associated with histones in a nucleosome, the specific binding-sites could be recognized and bound specifically by GR. In contrast to what appears to be the case in vivo, the nucleosome was not displaced as a result of GR-binding.

In other experiments the affinity of GR-binding specific sites in the MMTV promoter was compared with the binding affinity for nonspecific DNA. GR binds with rather low selectivity to specific binding sites. The affinity for specific sites in nucleosomal DNA was nearly as high as for sites in histone-free DNA. In contrast, the affinity for non-specific DNA organized in nucleosomes was drastically decreased compared to histone-free DNA, and may therefore help to explain how this regulatory molecule effectively can select the specific DNA binding-sites within the genome.

The role of chromatin has been further analyzed by nuclear injections into Xenopus oocytes. In these experiments, the assembly of nucleosomes was blocked in injected MMTV plasmids by coinjecting nonspecific competitor DNA to titrate endogenous histones. This resulted in a drastic increase in basal MMTV transcription. The hypothesis, that a nucleosome maintains the low basal level of the promoter, is supported by these results.

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Myocardial toxicity of antineoplastic drugs—Effects of daunorubicin singly and in combination with cyclophosphamide or ifosfamide evaluated by nuclear medicine, pathology and biochemistry—An experimental study in rabbits

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The cardiotoxicity of daunorubicin was evaluated a) after a single high dose (18–26 mg · kg^{-1}). b) after 2 weeks' treatment with 2.6–3.0 mg · kg^{-1} twice weekly and c) after long-term treatment with repeated low doses (2.25–2.6 mg · kg^{-1} · wk^{-1} × 10–13 weeks). The cardiotoxic effects of drug combinations were evaluated after completed long-term treatment with daunorubicin (2.25 mg · kg^{-1} · wk^{-1} × 10 weeks) followed by high-dose cyclophosphamide (200 mg · kg^{-1} over two days) or ifosfamide (300, 400 and 600 mg · kg^{-1} over two days.) Also the cardiotoxic effect of high-dose cyclophosphamide as single drug (100 and 200 mg · kg^{-1} over two days) was studied. The cardiotoxicity was evaluated by myocardial accumulation of ^{99m}Tc-pyrophosphate and ^{99m}Tc-gluconate, and by morphology. Myocardial accumulation of ^{99m}Tc-pyrophosphate was correlated with histopathologic changes and the myocardial accumulation of ^{99m}Tc-gluconate was correlated with the release of lactate dehydrogenase from isolated neonatal heart cells in culture.

Rabbits killed 6–7 h after a single high-dose of daunorubicin showed slightly increased myocardial uptake of both radionuclides. There were no morphologic changes. Rabbits receiving two weekly doses for two weeks had high myocardial uptake of ^{99m}Tc-pyrophosphate. No or discrete morphologic changes were observed. Long-term treatment with daunorubicin induced morphologic changes characteristic of chronic anthracycline cardiotoxicity. The ^{99m}Tc-pyrophospahte uptake was increased in rabbits killed one week after last daunorubicin dose, but declined in rabbits killed two weeks after last daunorubicin dose.

Rabbits treated with only cyclophosphamide had infrequent small foci of acute myocardial necrosis. Increased ^{99m}Tc-pyrophosphate uptake was not demonstrated in this group.

Rabbits treated with high-dose cyclophosphamide after completed daunorubicin showed enhanced cardiotoxicity with frequent myocardial necrosis and high isotope uptake compared with the rabbits receiving treatment with only daunorubicin or only cyclophosphamide. The toxic effect was probably synergistic. Rabbits treated with different high-doses of ifosfamide following daunorubicin showed a relationship between the dose of ifosfamide and the cardiotoxic effects.

Acute myocardial necrosis appeared to be essential for substantial isotope binding. Slightly increased isotope binding possibly occurred in myocytes with degenerative changes. Fibrosis did not accumulate radionuclide. Scintigraphy of entire heart preparations post mortem had a high sensitivity and the specificity for the detection of acute myocardial necrosis. Increased ^{99m}Tc-gluconate binding to isolated neonatal heart cells treated with daunorubicin corresponded to release of lactate dehydrogenase from the myocytes and to ceased beating of the cells.

The results indicate that high-dose cyclophosphamide and ifosfamide close to discontinued high cumulative therapy with anthracyclines should be considered as a risk factor cardiotoxicity also in human therapy. ^{99m}Tc-pyrophosphate and ^{99m}Tc-gluconate may be used in the experimental screening of agents for acute cardiotoxicity (acute necrosis). Myocardial scintigraphy with these radionuclides is less useful to follow experimental chronic anthracycline cardiomyopathy.

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Pharmacokinetics, hepatotoxicity, and drug interactions of methotrexate and 7-hydroxymethotrexate

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This dissertation deals with the hydroxylation of the antifolate methotrexate (MTX) to its major extracellular metabolite 7-hydroxymethotrexate (7-OH-MTX). In humans, high-dose MTX (HD-MTX) anticancer therapy results in up to millimolar serum concentration of 7-OH-MTX. The metabolite is highly insoluble in aqueous solution, and has been implicated as a possible mediator of potentially fatal renal failure during HD-MTX therapy. Acute MTX-associated hepatotoxicity has frequently been observed after HD-MTX infusions, and 7-OH-MTX has been postulated to be the culprit. However, the underlying mechanisms still remain unresolved.

We have shown that MTX hydroxylation is present in Wistar rats in vivo, and that this animal is suitable as a model for studies of the pharmacokinetics of 7-OH-MTX and the interactions of 7-OH-MTX formation in vivo. In this rodent MTX demonstrates dose-dependent kinetics as biliary secretion approached saturation

at drug doses exceeding 50 mg/kg, whereas 7-OH-MTX formation and excretion are not prone to saturation.

The 7-hydroxy metabolite at high biliary concentrations precipitates in alkaline rat bile. In vivo there is precipitate formation at biliary metabolite levels of approximately 9 mM, and this maximum solubility threshold can be reproduced by addition of 7-OH-MTX to freshly collected rat bile. Further experiments indicated that 7-OH-MTX possesses a role in the precipitate formation in rat bile following HD-MTX therapy and that it may be involved, at least in part, in the acute MTX-associated hepatotoxicity, a dose-limiting toxicity that may not be counteracted by leukovorin rescue.

The production of 7-OH-MTX may, however, be effectively inhibited in vitro and in vivo by two other anticancer agents, vindesine (VDS) and amsacrine (mAMSA). VDS acts by inhibition of the hepatocellular MTX uptake, whereas mAMSA acts by inhibition of MTX hydroxylating enzyme system, liver aldehyde oxidase. But whether VDS or mAMSA, by reducing the 7-OH-MTX, may increase the therapeutic index by modulating the clinical toxicity of MTX requires further investigation.

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