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.

Tumor necrosis factor and multidrug resistance—Biological properties of tumor necrosis factor and the relationship to multidrug resistance

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The following statements based on this work are made by the author:

Secretion of tumor necrosis factor (TNF) involves passage through secretory vesicles, and thus seems to be typical of secretory proteins.

TNF may exist in a membrane-anchored form, possibly as a transmembrane protein. This membrane-anchored form of TNF is biologically active.

Internalization of TNF is probably not required for TNF to induce biological effects.

TNF is not constitutively produced in vivo in circulating monocytes.

There is a positive correlation between emergence of multidrug resistance (MDR) and TNF resistance, but the mechanisms seem not to be directly coupled.

The ability to reverse MDR is a common property of lipophilic drugs, and this ability is additive.

1990

Radiation-induced lung injury—Biological and clinical studies in patients with thoracic malignancies

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Normal tissue tolerance limits radiation dosage and/or treatment volume in radiotherapy. In thoracic irradiation the lung is a dose-limiting tissue. The acute and late phases of radiation lung injury are well characterized clinically, although the mechanisms leading to these conditions are unclear. Most studies evaluating responses to radiation in lung tissue have been experimental. With the emergence of these new imaging techniques (CT, MR, and BAL) it has become possible to study radiation lung injury in humans.

During 1977–1988, a total of 100 patients with pleural mesothelioma were seen at the Department of Pulmonary Medicine, Helsinki University Central Hospital. Forty-six of these patients formed the study population for this investigation. The patients were treated by debulking surgery, chemotherapy and hemithorax irradiation. The radiotherapy was given according to 4 different consecutive schedules. One of the radiotherapy schedules used a low dose, 20 Gy, and the other schedules used high doses, 55 Gy, 70 Gy and 35 + 36 Gy.

The observed radiologically-assessed final effects of high-dose (55, 70, 35 + 36 Gy) hemithorax irradiation are compatible with a total loss of lung function on the irradiated side. Six months after the end of treatment all patients in these treatment groups had moderate to severe radiation injury, and all high-dose groups included individuals who at this stage had no normal aerated lung tissue left on the irradiated side. Only the low-dose group (20 Gy) showed less radiation lung injury. In addition, chemotherapy may have potentiated the radiation injury.

The extent and time-course of the lung injury caused by radiation could be defined by serial chest radiographs alone. Although CT detected the developing injury earlier and more precisely, it provided no cost effective diagnostic advantage over conventional x-rays in the detection of radiation pneumonitis of fibrosis. However, the documentation of tumour status and/or infections needed additional imaging or laboratory investigation, especially when lung injury was severe. The possible advantage of MR over CT could not be evaluated and needs further investigation. The optional time to detect the early signs of radiation lung injury following high-dose hemithorax irradiation by CT or MR were during the latter part of the treatment or very soon after the end of irradiation.

For research protocols evaluating radiation lung injury serial chest x-rays are recommended before treatment and 2, 6 and 12 months after treatment, with additional CT scans as required for differential diagnosis.

In contrast to previous data, both FVC and DL_{CO} showed a significant decline 1.5–2 months after the end of hemithorax irradiation and thereafter up to the end of the 1-year follow-up period. This may imply that hemithorax irradiation resembles pneumonectomy; the other radiotherapy regimens with irradiation of partial lung volumes may resemble lobectomy or segment resection. Neither FVC nor DL_{CO} could, however, be correlated consistently with the radiologically-assessed pulmonary changes, which is in accordance with previous data. Hypoxemia and pathologic physiologic shunting increased transiently 1–2 months after irradiation in 2 of 6 patients monitored; this phenomenon might be studied further. Before hemithorax irradiation, lung function should be evaluated as for pneumonectomy.

Following hemithorax irradiation: a) the concentrations of the surfactant components in ELF decreased to 12–55% of the control value prior to irradiation; b) the concentration of sphingomyelin in ELF increased 9-fold; c) the concentration of soluble protein in ELF increased from 8 to 57 mg/ml; and d) BAL fluid supernatant from irradiated lung showed an inhibitory effect on normal surfactant. There was significant correlations between increasing severity of radiological change/deterioration in lung function and the saturated phosphatidylcholine/sphingomyelin ratio, the concentration of soluble protein and the concentration of the major surfactant components in ELF. The distinct changes in surfactant concentration and in its biophysical properties shown in this study support the theory that the pneumocyte type II cell may be a primary target cell in radiation-induced lung injury.

The serum procollagen type III peptide level, measured weekly during the 5 weeks of radiotherapy, did not show consistent changes nor did it correlate with the final radiation fibrosis. Serum procollagen type III peptide measurement was not therefore found useful as an early marker or radiation fibrosis. Neither did the BAL fluid findings before or after treatment provide evidence that fibronectin or markers of plasminogen activation could be used as predictive markers. However, zymographic analysis of BAL fluid plasmin and plasminogen activation showed a tendency to be elevated in the patients experiencing severe readiation reaction. This suggests a possible role for inhibitors of plasminogen activation cascade in lung injury caused by radiation.

This study demonstrates that it is feasible to study radiation injury in human lung in the model of hemithorax irradiation for pleural mesothelioma. With the established assessment schedules

it is now possible to study patients following thoracic radiotherapy. In particular, studies with radiation response modifiers may provide new scientific and therapeutic possibilities.

November 1990

Significance of glutathione transferases in the resistance of tumor cells to alkylating cytostatic drugs

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The occurrence of various forms of cytosolic glutathione transferase (EC 2.5.1.18; GSTs) in a large number of human tumor cell lines derived from different tissues as well as in melanoma solid tumors, has been investigated. Furthermore, specific activities of other glutathione (GSH)-linked enzymes have been determined. Several lines of evidence indicate that most of the total GST activity in these samples (measured with 1-chloro-2,4-dinitrobenzene) was due to elevated levels of the class Pi isoenzyme (GST-π). Affinity chromatography of the cytosolic fraction of several human cell lines, followed by SDS-PAGE and immunoblotting revealed that typical class alpha and Mu enzymes are also present in malignant cells, but at lower levels than GST-π.

Using class-specific antibodies directed towards the various isoenzymes from rat, it was demonstrated that additional class alpha and Mu forms are expressed in human tumor cells. These forms are similar to those less frequently found in normal tissues and/or different from the classical human enzymes characterized previously. These results suggest that this is a useful approach for analysis of different tissues from rat and man.

One of the most significant observations in this study is the finding that every cell line studied displays a unique pattern of GSH-linked enzymes, regardless of its histological origin. In light of the possible involvement of the GSTs in antitumor drug resistance, this differential expression may be of clinical relevance.

The role of GSTs in cellular resistance to certain alkylating drugs used in the treatment of cancer has been investigated. As demonstrated by immunoblotting analysis using isoenzyme-specific antibodies, 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU)-resistant rat gliosarcoma 9L-2 cells show an altered pattern of GSTs as compared to the drug-sensitive 9L parental cells. Elevations of the levels of subunits 3 and 4 were the most prominent features of the new phenotype. Consistent with these findings, it was demonstrated that a GSH-dependent denitrosation reaction which inactivates BCNU is most effectively catalyzed by the class Mu isoenzymes containing subunit 4 (GST 4-4 > GST 3-4). In addition, pretreatment with the GST inhibitors ethacrynic acid and triphenyltin chloride caused a significant reduction in the degree of resistance to BCNU. Thus, the isoenzyme-dependent inactivation of BCNU and the up-regulation of class Mu GST subunits appear to contribute to the increased resistance of 9L-2 cells.

Despite the relatively high degree of resistance to BCNU or nitrogen mustard demonstrated by 9 glia-derived human cell lines, neither the levels of GSTs nor the contents of GSH and protein-thiols could be related to this resistance. On the other hand, depletion of cellular GSH by pretreatment with buthionine-S,R-sulfoximine sensitized the cells to the cytotoxic effects of BCNU and nitrogen mustard.

Finally, it was found that RPMI 8322 human melanoma cells, which show inherent resistance to melphalan and contain a broad spectrum of GST isoenzymes, were potentiated to the cytotoxic effects of this alkylating drug by pretreatment with ethacrynic acid. In support of these and earlier results, it was shown that the strength of inhibition of the catalytic activity of purified human isoenzymes by ethacrynic acid is GST-$\mu \gg$ GST-$\varepsilon >$ GST-π.

In summary, the experimental data support the hypothesis that GSH and GSTs are significant factors contributing to cellular resistance to alkylating drugs.

In addition, a summary of the literature concerned with the topics of this study is presented.

January 1991

Radiosensitization by oxygen and radioprotection by thiols—Experimental evaluation of a theoretical model

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The general purpose of the investigations presented in this thesis was a further elucidation of the mechanism by which oxygen sensitizes cells and tissues to radiation. A closer knowledge of this mechanism is, besides a theoretical, of a particular practical interest in view of the well recognized importance of the effect of oxygen in the radiotherapy of tumors. Recently, the 'X model' was put forward as an explanation for the oxygen effect. The model postulates that oxygen and cellular thiols compete for radiation induced target radicals, oxygen fixing and thiols repairing the radiation damage, the latter, however, with the reservation that only a certain proportion of the damage is reparable. The study summarized in the thesis was performed with the aim of testing the validity of the model by a comparative analysis of the theoretical predictions with experimental observations.

Human fibroblasts and Chinese hamster cells in culture were used as experimental cell material. The thiol level of the cells varied either due to a genetic damage, or to treatment with buthionine sulphoximine (BSO) which decreased the thiol content, or to treatment with dithiothreitol or N-acetylcysteine which increased it. Exposures to radiation were made in severe hypoxia or in the presence of oxygen in varying concentrations. The yield of DNA breaks, expressing the effect of the initial radical reactions and, in a few cases, clonogenic survival, expressing also the effect of additional superimposed enzymatic repair processes, were chosen as measure of the radiation response. Four different predictions of the X model were tested experimentally. In all cases a good general agreement between the patterns of the radiation response, predicted by the X model, and the experimental observations was found. In contrast, major differences were noted between the experimental data and the prediction of an alternative model in which, as opposed to the X model, radiation-induced radical damage is assumed to be reparable by thiols in full. The validity of the X model, indicated by these investigations, suggests that in general two types of damage are produced by radiation, only one of which can be repaired by thiols.

March 1991

Tumor markers and adenocarcinomas in colon and pancreas

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CA 19-9 and CA 50 were studied in 72 patients with pancreatic cancer. Their value as serological tumor markers was confirmed. With higher cut off levels than those commonly used, we obtained a sensitivity of more than 80% and high specificity for both

markers. Furthermore, the markers were useful for follow-ups after surgery.

Two polyclonal and two mouse monoclonal antibodies were investigated for their efficacy in radioimmunodetection (RAID) experiments. Nude mice with transplants of the human colon carcinoma cell line LS 174 T were injected with ^{125}I labels of the antibodies. The monoclonal antibody (Mab) 38 S1 showed superior tumor localization. This was also the case in later clinical trials.

Ten patients with advanced colorectal cancer received therapy with the mouse monoclonal antibody 17-1A (Mab 17-1A) and were investigated for the tumor markers CA 19-9 and CA-50. They yielded valuable information; a decrease in the serum concentrations of these tumor markers served as a criterion for response in the evaluation of the patients undergoing Mab 17-1A therapy.

Patients receiving Mab therapy were also investigated for the alkaline phosphatase isozymes; the unspecific alkaline phosphatase (LAP), the intestinal alkaline (IAP), the placental alkaline phosphatase (PLAP) and the PLAP-like isozyme. Highly elevated serum levels of the PLAP-like enzyme were detected in the patients, as described earlier primarily in patients with seminoma and ovarian cancers. The elevations followed tumor progression. A catalytic assay showed that the enzyme was partly heat labile, in contrast to the PLAP-like enzyme normally found in tumors. Further characterization of the alkaline phosphatases in colorectal adenocarcinoma tissues and normal colorectal mucosa tissues was carried out. A changed isozyme pattern in the cancers compared to the normal mucosas was detected, with elevations of the LAP and the PLAP-like isozymes. Using amino acid inhibitions and immunoblotting tests, the latter enzyme was confirmed to be of the PLAP-like type.

Three potential epitopic sites were detected in the PLAP-like molecule. Three monoclonal antibodies, reactive with the PLAP-like enzyme, were used for peptide mapping. The epitopic sites for two of three Mabs were discontinuous.

A heterogeneity of tumor marker populations exists, with varying immunological and biochemical properties. Characterization of tumor markers in experimental studies to improve their efficacy in the clinic is therefore of importance.

March 1991

Tumor markers for ovarian carcinoma—Diagnostic and prognostic studies with emphasis on the CA-125 antigen

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In the present investigation the CA-125 antigen was explored as a diagnostic indicator for ovarian carcinoma, alone, or in combination with CEA (Carcinoembryonic antigen), TPA (Tissue polypeptide antigen), or PLAP (Placental alkaline phosphatase). Pre-treatment serum samples were obtained from 295 women with adnexal masses. The prevalence of malignancy was 65%. The majority of the women were postmenopausal.

The sensitivity of the CA-125 antigen for ovarian carcinoma was 88%, for limited disease 74%, and for borderline tumors 58% (cut-off 35 U/ml). Only 35% of the mucinous cases were detected. However, 15/17 mucinous cases had abnormal CEA levels. The combination of CA-125 and CEA (cut-off 15 μG/l) was most efficient for discrimination between malignant and benign adnexal masses. The sensitivity for ovarian carcinoma was 93%, for limited disease 83%. Specificity was 83%. Predictive value of a positive test was 90% for malignancy, that of a negative test was 76% for benign disease.

Multiple regression analysis showed that the CA-125 level was dependent on FIGO stage in ovarian carcinoma, but only in non-mucinous cases. Histological grade had no influence. The CEA level was dependent on clinical stage in mucinous cases only. CA-125 was detected immunohistochemically in the epithelium of benign, borderline, and malignant non-mucinous tumors, CEA in mucinous variants. The correlation with corresponding serum levels was strong in invasive carcinoma. In borderline, and particularly benign cases CA-125 levels were low despite positive immunostaining, indicating the presence of compartment barriers. Evidence was found in benign tumor cases, that ascites or secondary peritoneal response is a non-specific cause of 'false positive' CA-125 levels.

Tissue expression of CA-125 or CEA in ovarian carcinoma could not be related to histological grade, DNA ploidy or S-phase rate, nor patient survival. The DNA profile had independent prognostic value, however.

In a simple graphical model, it was shown that the sensitivity of a diagnostic test is dependent upon the characteristics of the population under study. The present results could be compared with data from other studies in this model. A future 'relevant' sensitivity for CA-125 was estimated.

In elderly or middle aged women with adnexal masses the CA-125/CEA combination of serum tests provides clinically relevant information for the detection of malignant disease, and deserves further evaluation in the preoperative diagnosis of ovarian carcinoma.

March 1991

Dose calculation methods in photon beam therapy using energy deposition kernels

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The problem of calculating accurate dose distributions in treatment planning of megavoltage photon radiation therapy has been studied. New dose calculation algorithms using energy deposition kernels have been developed. The kernels describe the transfer of energy by secondary particles from a primary photon interaction site to its surroundings. Monte Carlo simulations of particle transport have been used for derivation of kernels for primary photon energies from 0.1 MeV to 50 MeV. The trade off between accuracy and calculational speed has been addressed by the development of two algorithms; one point oriented with low computional overhead for interactive use and one for fast and accurate calculation of dose distributions in a 3-dimensional lattice, however with a greater overhead. The latter algorithm models secondary particle transport in heterogeneous tissue by scaling energy deposition kernels with the electron density of the tissue. The accuracy of the methods has been tested using full Monte Carlo simulations for different geometries, and found to be superior to conventional algorithms based on scaling of broad beam dose distributions. Methods have also been developed for characterization of clinical photon beams in entities appropiate for kernel based calculation models. By approximating the spectrum as laterally invariant, an effective spectrum and dose distribution from contaminating charged particles are derived from depth dose distributions measured in water, using analytical constraints. The spectrum is used to calculate kernels by superposition of monoenergetic kernels. The lateral energy fluence distribution is determined by deconvolving measured lateral dose distributions by a

corresponding pencil beam kernel. Dose distributions for contaminant photons are described using two different methods, one used to estimate the dose outside of the collimated beam and the other for calibration of output factors derived from kernel-based dose calculations. The beam characterization methods have been carefully tested in several clinical beams, showing good agreement of calculated and measured dose distributions.

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Radiation therapy planning and optimization studied as inverse problems

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Optimization procedures are vital in all radiation therapy activities. The main task is to ensure that a sufficiently high dose is delivered to the tumor cells while keeping the dose to normal tissues as low as possible. The optimization process can be studied mathematically as an inverse problem if the dose distribution delivered during radiation therapy can be formulated analytically. In general the prescribed dose distribution can be built up in terms of a focused mean specific energy deposition kernel that can be applied to all parts of the tumor as described by a local irradiation density. This approach is also well suited for optimization of the treatment, for example in terms of the probability of achieving complication-free control of tumor growth. The biological properties of tumors and normal tissues can then be used to optimize the treatment instead of prescribing an estimated optimal dose distribution. The inverse problem of finding the optimal incident beams is here solved by numerical algorithm utilizing Fast Fourier Transform techniques. The same algorithm is also well suited for solving the problem of generating highly non-uniform incident dose profiles necessary to fully exploit the power of the optimization method.

April 1991

Clonal B cells and immunoregulatory functions in monoclonal gammopathies—An in vitro immunological study

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Clonal B lymphocytes and immunoregulatory cell populations were studied in patients with monoclonal gammopathies (MG).

The tumor cell clone in MG is generally believed to comprise plasma cells in the bone marrow, but may also include peripheral blood B lymphocytes bearing identical idiotypic (id) immunoglobulin (Ig) surface structures as in the cytoplasm of the plasma cells. Id-bearing B cell clones were established from the peripheral blood of a patient with MG by immune rosetting and cloning by limiting dilution. Ten Ig-bearing clones specifically bound an anti-id antibody as assessed by immunofluorescence and ELISA. DNA from three clones had Ig gene rearrangement patterns identical to the specific one in the bone marrow.

The presence of clonal B cells in the peripheral blood of patients with untreated multiple myeloma was found to be a strong predictor of prognosis.

NK cells might have a regulatory impact on normal B lymphocytes. It might be anticipated that NK cells may have a regulatory function also on malignant B cells. As a first attempt to characterize NK cells in human MG, NK cell functions in the peripheral blood of MG patients were studied and related to disease activity. A high NK activity and high numbers of cells with NK related cell surface markers were found in patients with a low tumor burden, whereas the NK functions were low in patients with advanced disease.

An id-specific cellular and humoral immunity with regulatory influence on the myeloma tumor cell clone have been demonstrated in murine myeloma systems. Here, id-specific immunity in human MG was studied.

The production of anti-id antibodies was analysed from cell cultures of EBV-transformed peripheral blood lymphocytes of MG patients. A high anti-id production was found in patients with a low tumor burden. In patients with advanced disease, the anti-id production was low.

The presence of id-binding T cells as well as T cell reactivity with a panel of TCR anti-V gene specific MAbs was studied. Three out of 11 tested patients had 1–15% id-binding $CD4^+$ or $CD8^+$ T cells in the peripheral blood. Three patients had a biased TCR V gene expression, but these T cell populations did not bind the id. T cell clones were isolated from repeatedly id-stimulated cultures of peripheral blood mononuclear cells from three patients with MG. In all three patients $CD4^+$ or $CD8^+$ T cell clones were obtained which bound to and showed a significant proliferative response to autologous id Ig.

These results provide further support for MG as differentiating B cell disorders. The cellular and humoral immunity against id Ig structures as well as NK cells might have a regulatory role on the tumor cell clone in MG.

April 1991

On the interferon system in primary human tumor cells

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Treatment with interferon (IFN) can induce remissions in various malignancies. How IFN exerts these antitumor effects is not known, but several mechanisms have been suggested. The studies have dealt with various aspects of the interferon system in primary human tumor cells:

- In vitro effects of IFN on myeloma cells.
- The IFN system in acute lymphocytic leukemia (ALL) cells.
- Development of a predictive test for IFN therapy.

By the use of a dye exclusion assay, IFN was found to significantly decrease (sometimes by $>90\%$) the number of viable malignant cells from patients with myeloma. These cells are almost exclusively non-proliferating in vitro. Depletion of autologous T-cells, NK-cells or macrophages did not abrogate the effect observed. It is concluded that IFN exerts a direct cytotoxic effect on primary human myeloma cells, unrelated to cell growth inhibition.

IFN was also found to significantly reduce monoclonal immunoglobulin (mlg) production in myeloma cells from 27/27 patients. There was, however, no correlation between decreases in myeloma cell viability and reduction in mlg production, i.e. mlg production was reduced also in patients showing no sensitivity to IFN's cytotoxic action. Thus, IFN may in some cases decrease mlg production without exerting a 'true' antitumor effect.

Malignant cells from 16% of patients with ALL were found to have complete or hemizygous deletions of the α/β-IFN locus. Other aspects of the IFN system, such as IFN-production, α-IFN receptors and IFN induced enhancement of the enzyme 2',5'-

oligoadenylate synthetase (2',5'-A synthetase) were studied in parallel. In total, 63% of the clones showed some abnormality in their IFN system, i.e. deleted IFN-genes, reduced IFN-producing capacity or reduced sensitivity to IFN. This finding adds some support to the hypothesis that defects in the IFN system could be a step in the pathway to malignant transformation in ALL. Clones with deleted IFN genes were in all cases studied found to have retained sensitivity to IFN, indicating that these patients may constitute a subgroup of ALL with a greater likelihood of response to IFN therapy.

An assay was developed to test IFN sensitivity in primary tumor cells from various solid tumors, measuring the capacity of IFN to induce 2',5'-A synthetase. Different tumors varied significantly in their sensitivity to IFN. The test is rapid (48 h), requires few cells and is not dependent on in vitro growth. The assay can also be performed using tumor cells from fine-needle aspiration biopsies. To determine the predictive value of the assay, a study was performed in tumor cells from 21 previously untreated patients with metastatic mid-gut carcinoid. IFN achieved a clinical response by predetermined criteria in 9 out of 21 patients (43%). There was a signficant correlation between induction of 2',5'-A synthetase in vitro and clinical response. The results indicate that a 2',5'-A synthetase assay can be used as a predictive test for IFN therapy in mid-gut carcinoid and possibly also in other tumors.

April 1991

Molecular studies of growth factors and their receptors in human gliomas

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Subversion of normal growth regulatory pathways is the mechanism by which tumours arise. This occurs by the mutation of genes whose products play key functions in regulation of cell growth and development. These processes have mostly been studied in culture systems, using tumour-derived cells. The aim of this thesis was to investigate whether similar findings could be demonstrated in human tumours in vivo. The gliomas, a group of brain tumours of neuroectodermal origin, which have been well documented to progress from subtypes of low malignancy grade, to highly malignant variants, were chosen as a model system. Three growth factor—growth factor receptor systems were studied in tumour biopsies from gliomas of all subtypes and malignancy grades. They were the following: a) The EGF-system: 66 gliomas were investigated for the quantity and quality of the genes and transcripts for EGF, TGF-α and EGFR. Tumours of all malignancy grades and subtypes co-expressed mRNA coding for both of the ligands as well as EGF-receptor mRNA. The possibility of an auto-, juxta- or paracrine growth stimulatory loop in these tumours is thus a reality. Furthermore, the gene for EGFR was found to be amplified and over-expressed in 50% of the most malignant form of glioma, glioblastoma. EGFR amplification only occurred in one of 30 low-grade gliomas studied, indicating a role for EGFR in the late stages of glial tumour progression. In about half of the cases with EGFR-gene amplification, rearrangements were detected. In a selected group of tumours with EGFR-gene amplification—detailed study showed variable rearrangements of the 5'-end of the gene which codes for parts of the extracellular domain of the receptor protein. The gene rearrangements all gave rise to an identical mutant mRNA lacking 801 bases (but remaining in frame), due to the aberrant splicing of exon 1 to exon 8 in each case. These 801 bases code for extracellular sub-domain I and part of sub-domain II. This mutation may abolish ligand binding and render the receptor constitutively activated. In a further, but more uncommon, group of glioblastomas, the EGFR-gene rearrangements clustered to the parts of the gene coding for the cytoplasmic domain. Again the deviant transcript arose by aberrant splicing. Two variants were observed, both of which lacked the sequence coding for the domain responsible for internalization of the ligand activated receptor. One of these splice variants lacked, in addition, sequence coding for the C-terminal tail which contains further control elements. The loss of these regulatory sequences may also result in an over-active receptor. Some tumours co-expressed both the extracellular—and the intracellular-mutations indicating an oncogenic progresson of the EGFR gene towards and v-erbB-like molecule in human primary glioblastomas. b) The EGF system. The mRNA and protein expression of aFGF was investigated in 60 human gliomas. An inverse relationship between aFGF expression and malignancy grade was noted, where glioblastomas expressed the lowest levels of mRNA for this angiogenic protein. The aFGF protein was localized by immunofluorescense to vascular endothelium and to single axons. In addition, tumour-tissue co-expressed variable levels of FGF-R mRNA indicating the possibility of FGF-mediated auto-, juxta- or paracrine growth stimulatory loops in gliomas.

c) The PDGF-system: The wild type gene for the PDGF-α-receptor was found to be amplified and overexpressed in a human glioblastoma. Co-expression of ligand also presents the possibility of autocrine growth stimulation. In conclusion, all growth factor-growth factor receptor systems studied are seriously disturbed in primary human gliomas and this is likely to contribute to their malignant phenotype.

April 1991

Xenografted squamous cell carcinoma of the head and neck—A tumour model for studies of drug effects

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Experimental models of human or animal cells growing in vitro or in vivo are important to improvements in cancer treatment. The present studies were designed to test such a system, where the effects of chemotherapeutic agents and surgery were studied in xenografts of human squamous cell carcinomas of the head and neck serially passaged in nude mice.

Cisplatin and fluorouracil given at 14.00 or 02.00 h, when circadian variation in cell cycle phase distribution was most pronounced, did not differ in toxicity or tumour growth inhibition.

One tumour line manifested decreased growth rate with increasing passage. After over 100 serial passages in nude mice, there was also a sudden increase in sensitivity to cisplatin. Only one of two different cell types remained after passage 108.

Tumour growth inhibition and toxicity of the cisplatin-fluorouracil combination was schedule-dependent, the optimum being fluorouracil given three days after cisplatin.

Verapamil did not enhance the tumour growth inhibiting effect of cisplatin, as tested with three different schedules.

Both after sham operation and subtotal resection of one of two tumours, the S-phase fraction in the contralateral, unoperated tumour decreased 24 h postoperatively. Residual tumour S-phase fraction did not change with time.

The acid test of treatment is its clinical effect, but as for

practical or ethical reasons certain questions are difficult or impossible to answer in clinical trials, the nude mice model of human tumour xenografts is an important asset.

April 1991

Human somatic hprt mutation and genetic toxicity of acetaldehyde

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Acetaldehyde (Aa), the first metabolite of ethanol oxidation, which has a wide spread occupational and environmental occurrence, and has shown clastogenic, teratogenic and carcinogenic effects in animals, was studied with regard to its ability to induce DNA damage, sister chromatid exchange (SCE) and mutation at the X-linked gene for hypoxanthine guanine phosphoribosyl transferase (hprt) in human lymphocytes in vitro. The human T-cell cloning technique was established in order to select for TG-resistant cells, and characterize hprt mutation at the molecular level.

Aa was found to be an efficient inducer of SCE in human peripheral lymphocytes. Results from treatments in different phases of the cell cycle indicated that the SCE-inducing effect of Aa is most efficient in cells close to S-phase and has a remarkable stability in cycling cells as well as in resting cells under continuous presence of Aa. New SCEs were formed in the second and even the third cell cycle after Aa removal. These results suggest that Aa may accumulate in the cells, possibly by forming reversible Schiff bases, and when released give rise to SCE-inducing DNA damage. The results from alkaline elution experiments indicated that Aa induces DNA cross-links in human lymphocytes which are likely to be responsible for at least part of its SCE-inducing effect. Using the T-cell cloning technique, Aa was found to induce a 3–16 fold increase of the mutant frequency at the hprt locus. Southern blot analysis of 41 mutant clones showed a predominance of large 3′-flanking deletions suggesting that this may be a major type of Aa-induced hprt mutation. These results indicate that there may be a common pathway through recombinational removal of DNA cross-links, for the generation of Aa-induced gene mutation, SCE and chromosome aberrations.

In vivo derived T-cell mutants were obtained from healthy subjects and melphalan-treated ovarian carcinoma patients. Southern blot analysis of 108 mutants showed gross structural alterations of the hprt gene in 14% of the clones from healthy males. The frequencies in the patients and female controls were found to be lower, probably due to the presence of the inactive X-chromosome which makes alterations more difficult to detect. Total as well as 5′-,3′-flanking and internal deletions with breakpoints in introns 1, 2 and 3 were found. Six hprt mutant clones were further studied by direct sequencing of PCR (polymerase chain reaction)—amplified hprt cDNA. In addition to a genomic deletion of a gene segment containing exons 2 and 3, three different base substitutions in the coding region were identified as responsible for two missense and one nonsense mutations. Two putative splice mutations, causing deletion of exons 2 + 3 and 8 respectively, were also observed.

These results demonstrate the diversity of somatic in vivo mutation at the human hprt locus.

April 1991

Quality assurance of simulator and verification imaging in radiation therapy

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The principal goal of radiation therapy is to ensure that the correct region of the patient receives the prescribed treatment and that the tissues surrounding the diseased region are spared. Tumour volume, target volume and organs at risk must be accurately delineated to obtain accurate dose distribution. Uncertainties resulting from the beam set-up and physical constancy used in calculations of treatment planning algorithms are usually of systematic nature; uncertainties resulting from patient set-up and patient motion are more likely to be of random nature. It has been shown that by increasing the precision of the delivery dose, the cure of early stage patients can be increased at a rate of about 2% per 1% improvement of accuracy.

Transferring the patient from the simulator to the treatment machine is a difficult step which entails more serious position errors than the actual day-to-day treatment reproducibility. This indicates the importance of portal films. A cassette of stainless steel was therefore specially constructed at the radiation therapy department. This cassette, together with Kodak TL film, resulted in portal film image quality with high contrast and good resolution.

When the quality of the portal films made it possible to recognise anatomical structures the reproducibility of radiation field alignment could be studied and the set-up accuracy determined. Thirty-five patients were included in the study, 642 portal films were taken. The mean standard deviation was 3.5 mm.

Simulators are acquired by radiation therapy departments for two reasons: to provide precision treatment planning and to save valuable time on busy treatment machines. The geometrical parameters of the simulator must be identical with those of the treatment unit. A trained and experienced technician can be responsible for the simulator procedures.

Processors employed in radiation therapy departments are often adjusted to diagnostic films and machine load resulting in poor quality portal films. The quality of portal films can be largely improved by proper choice of film and cassette and by regular control of the processor.

The quality of our portal images are equal to and sometimes better than the quality obtained with the two tested digital devices. A major advantage of on-line portal imaging compared to film is that the images are available on a video display only a few seconds after the radiation beam has stabilized. On-line imaging devices will be mandatory for quality control purposes as new treatments such as flying wedge, multileaf collimators, scanning beam and computer controlled radiotherapy are being introduced.

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