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Abstracts of Theses from the Nordic Countries

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Studies of cell migration and matrix protease production in human lung cancer cell lines

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Metastatic spread in cancer is a complex multistep process involving continuous, sequential interactions between tumour cells and their respective microenvironment. Invasiveness per se has not been studied in this thesis, only some of its major components, such as cell migration and matrix protease production. To better understand some of these components, we used human lung cancer cell lines representing the four major histologic types: adenocarcinoma (Wart); squamous cell carcinoma (U-1752); small cell lung carcinoma (SCLC) (U-1906, 054A); and large cell carcinoma (U-1810).

Paper I concerns results of studies of cell migration using Boyden chamber assays. It was shown that cell lines Wart, U-1906, and 054A migrated chemotactically and haptotactically to components of the extracellular matrix–such as fibronectin, laminin and type IV collagen in a β1 integrin-dependent fashion. In contrast, cell line U-1752 did not respond chemotactically to any of the three extracellular matrix components that were used. Fibronectin and type IV collagen induced chemotactic and haptotactic migration of the U-1810 cells, but laminin did not. Our study confirmed variable expression of integrins belonging to the β1 family in human lung cancer cell lines, except in cell line U-1810 which did not express the β1 integrin subunit. The migratory response differed depending on whether the chemoattractant was in a soluble or an insoluble form.

Paper II deals with studies of the expression of different growth-factor receptors and corresponding ligands in human lung cancer cell lines. Using RT-PCR, we found that IGF II/M6P, c-met, EGF and c-kit receptors are expressed in 5/5 cell lines. In order to investigate the biological function of these receptors, we performed Boyden-chamber assays using various growth factors as chemoattractants. The main result in this study was the finding that IGF I, IGF II, HGF, EGF and SCF stimulate migration of human non-small cell lung carcinoma (NSCLC) cell lines in a dose-dependent manner. In addition, checkerboard analysis demonstrated both a chemotactic and a chemokinetic response–indicating that these growth factors may act in both an autocrine and a paracrine fashion in vivo.

In studies reported on in paper III, we used gelatine zymography to demonstrate that three NSCLC cell lines express matrix metalloproteases (MMPs) -9 and -2. The expression and activity of MMP-9 and MMP-2 was heterogeneous in these cell lines. In addition, we showed that growth factors modulate MMP activity. HGF and EGF are capable of stimulating the conversion of MMP-9 from a latent to an active form in human large cell lung cancer cell line U-1810. Furthermore, IGF I, IGF II, HGF, and EGF stimulated an enhanced expression and activity of the latent form of MMP-2 and MMP-9. SCF did not enhance MMP activity in any of the cell lines that were tested.

In the studies described in paper IV, we found that cross-linking of α2β1 on U-1752 cells with immobilized mAb induced motile behaviour in the absence of extracellular matrix components. In addition, it was shown that α2β1 triggered migration was enhanced by growth factors–such as EGF and HGF. α2β1 triggered migration could be blocked if the cells were pretreated with genistein, pertussis toxin or calphostin C, indicating the involvement of protein tyrosine kinases, G-proteins and protein kinase C-dependent signalling pathways, respectively.

September 2004

To lose a child to cancer—A nationwide study of parental experiences

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This thesis investigated the long-term psychological consequences for parents of losing a child to cancer and whether any care-related stressors affect the bereavement outcome. An evaluation of the parents' perception of participating in the study was also part of the aims.

Among all eligible 561 parents in Sweden who had lost their child to cancer 4 to 9 years prior to the study, 449 (80 percent) responded to an anonymous postal questionnaire as did 457 out of 659 (69 percent) non-bereaved parents serving as controls. The questionnaire assessed anxiety, depression, sense of well-being and self-assessed quality of life, as well as potential care-related stressors.

Still 4 to 6 years after the loss bereaved parents had an increased risk for psychological complications; the relative risk (RR) being 1.7 for both anxiety and depression. However, 7 to 9 years after bereavement, the risk approached the levels of non-bereaved parents. Within the bereaved group, parents losing a child 9 years old or older had an increased risk for anxiety (RR 1.5) and depression (RR 1.6) compared to those losing a younger child.

Four to nine years after the loss, as many as 57 percent of the bereaved parents were still affected by the child's unrelieved pain and difficult moment of death. The probability of the parents reporting the latter was increased (RR 1.4) if staff had not been present at this moment. None of the 147 parents who had talked about death with their child regretted it at follow-up. Among 258 parents who had not talked about death with their child, 69 (27 percent) regretted this decision 4 to 9 years after the bereavement. Regret was strongly related to parents having sensed that their child was aware of his or her imminent death (RR 3.7)

Most bereaved parents perceived the inquiry concerning their child's care and death as being valuable (423/427, 99 percent) and the majority reported that the study had affected them positively (285/421, 68 percent).

Bereaved parents are still at increased risk of psychological complications 4 to 6 years after the loss of their child, after which the risk subsides close to normal levels. Our study suggests that few, if any, bereaved parents regret having talked about death with their child. The child's unrelieved pain and difficult

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moment of death affect bereaved parents for a long period of time after bereavement. A postal inquire after the loss of a child to cancer can be perceived as valuable, and even positive, by the parents.

September 2004

Genetic susceptibility to breast and endometrial cancer

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Hormones are central in the carcinogenic process in the breast and in the uterine epithelium. Individual genetically determined variation in the response to hormonal influence may alter susceptibility to breast and endometrial cancers. Many small studies of this hypothesis have generated inconclusive results. Since the effect of any genetic variant is expected to be modest, large studies are needed to draw reliable conclusions. Also, there may be interaction between genetic and lifestyle factors that needs to be considered. To address these issues, we conducted a large population-based case-control study that incorporated genetic and lifestyle exposures. We investigated common variants in the genes for the estrogen metabolizing enzymes catechol-O-methyl transferase (*COMT*) and cytochrome P450 1B1 (*CYP1B1*) and in the estrogen (*ESR1*), androgen (*AR*) and vitamin D receptors (*VDR*), selected from previous literature, in relation to breast and endometrial cancer risk. We genotyped 1569 breast cancer patients, 707 endometrial cancer patients and 1729 partly shared population controls. All participants had previously provided extensive information via a questionnaire about lifestyle factors such as reproductive history, body size, and use of menopausal hormone preparations.

We found that a *COMT* allele that confers high enzyme activity (c.324/474A > GG) was associated with increased risk for lobular breast cancer, but not with breast cancer risk overall. The *CYP1B1* variants, c.355G > T, c.4326C > G, and c.4390A > G, were not associated with breast cancer risk. The common *ESR1* c.454-351A > G allele was associated with relatively higher cancer risk compared with the rare allele, although in breast cancer only when considered in a haplotype with c.975C > G. The endometrial cancer risk in women homozygous for the rare c.454-351A > G allele was just half of the risk in those homozygous for the common allele. The *AR* CAG_n and *VDR* A_n were not associated with breast cancer risk. In exploratory subgroup analyses stratified according to lifestyle factors, we found that in women with diabetes mellitus the high activity *COMT* allele was associated with an increased risk for breast cancer. In women who had used menopausal hormones for at least four years the *CYP1B1* c.4326C > GG allele conferred increased breast cancer risk. The association between the *ESR1* c.454-351A > G and c.975C > G AC haplotype and breast cancer was stronger among women with a BMI above 30. Women who carried two short alleles of *VDR* A_n had a halved risk for breast cancer, irrespective of parity.

The most persuasive association in this work was the similar relation between variation in *ESR1* and breast and endometrial cancer. A stronger association with endometrial cancer was expected due to its apparently lower degree of etiological complexity relative to breast cancer. The estrogen receptor is crucial in estrogen stimulation and thus the prior probability of this gene being linked to breast and endometrial cancer risk was high. The remaining results indeed add to the body of evidence regarding breast and endometrial cancer susceptibility but the statistically significant associations may well be due to chance.

October 2004

Pharmacology of low-dose tamoxifen regimens in breast cancer treatment

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Breast cancer (BC) is the third most common cancer in the world following lung and stomach cancers. It is the leading cause of cancer mortality in women, and accounts for 21–22% of all new cancer cases. The lifetime risk of BC diagnosis for females in Europe is approximately 13% (i.e. approx. 1 in 8 women). Worldwide, BC survival in the year 2000 was estimated to be about 64%. During the last 35 years, the incidence of BC has almost doubled in the Nordic countries, with the incidence in Denmark, Finland and Sweden being 75–80 cases per 100000 population. In sub-Sahara Africa, BC is the third common cancer after cervical cancer and Kaposi's sarcoma. A study of the Tanzanian national cancer registry (1968–1996) indicated that the incidence of female BC was 55–88 per 100000 population. Tamoxifen, a selective oestrogen receptor modulator, is widely used for the treatment of BC and the only drug approved for the prevention of this disease.

Long-term dosing provides greater therapeutic advantage, but may amplify toxic effects. Its oestrogen-agonistic properties cause serious side effects like endometrial cancer and thrombotic diseases, which may limit its use in healthy women. These adverse effects may be both time- and dose-dependent.

The clinical part of the thesis covered a comparison of the effects of two low-doses of tamoxifen (1 and 5 mg/day) with those of standard dose (20 mg/day) given for 28 days, on BC proliferation as assessed by the change in proliferation index Ki-67. In addition, we measured an array of circulating biomarkers of risk of BC and other diseases including insulin-like growth factor-I, and sex hormone-binding globulin (SHBG) that are modulated by tamoxifen.

The effects of lower doses of tamoxifen on Ki-67 expression were comparable to those achieved with the standard dose. There was linear pharmacokinetics even in the low dose range. Tamoxifen and metabolite levels in serum and tissues were inter-related, and were significantly associated with changes in serum SHBG levels. This suggests a retained antitumour activity and a lower endometrial stimulation by the lower doses. A higher variation of serum tamoxifen levels (> 10-fold) among subjects in the dose groups and the observed serum to tissue concentration relationships suggest that therapeutic drug monitoring (TDM) may be used to further optimize tamoxifen treatment. Lower doses of tamoxifen should be assessed further in randomized trials.

Studies on nude rats and nude mice investigated the metabolism of tamoxifen between the species. The oral and subcutaneous routes of tamoxifen administration, and changes in the expression of drug metabolising enzymes (CYP3A2, CYP3A18 and FMO1) were further examined in nude rats.

Unlike nude mice, tamoxifen metabolism in nude rats was more similar to that observed in human. An increase in CYP3A2, CYP3A18 and FMO1 mRNA expression levels was observed in the orally treated animals, but no significant enzyme induction

was observed in the subcutaneously dosed animals. Oral administration of low-dose tamoxifen in nude rats resulted in enzyme induction and a metabolite profile similar to that observed in man whereas subcutaneous treatment did not.

In conclusion, our data demonstrate that oral dosed nude rats are a preferable animal model for studying tamoxifen pharmacokinetics. Tamoxifen induces liver mRNAs coding for enzymes that produce and eliminate its active metabolites, a process that may cause a time lag before steady state concentrations are obtained. The complexity of tamoxifen metabolism and a high interindividual variability in blood levels suggest that inclusion of TDM in tamoxifen trials may increase the benefit and quality of such studies.

November 2004

Traditional or individualised follow-up in women after breast cancer surgery

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The general aim was to compare different follow-up approaches after breast cancer surgery, i.e. traditional follow-up to a physician and individualised approaches, with specific emphasis on satisfaction, well-being and self-care. Both quantitative and qualitative research methods have been employed. This thesis is based on two study cohorts; 264 women who had undergone surgery for breast cancer between 1991–2001 at two hospitals in Sweden were consecutively randomised to two parallel groups. From this study cohort 20 women were interviewed about their experience of traditional follow-up to a physician and 19 women were interviewed about their experience from the nurse-led follow-up on demand. The two systems; traditional physician follow-up and nurse led follow-up on demand were compared and evaluated. The needs of women after breast cancer surgery were explored. A new study cohort of 96 women who had undergone surgery for breast cancer and who were consecutively selected and divided into two parallel groups between 2001–2003 at two hospitals in Sweden were studied. Traditional physician follow-up and a multi-disciplinary educational programme were compared. Instruments such as The Hospitality Anxiety and Depression-scale, the Functional Assessment of Cancer Therapy-General scale and the Sense of Coherence scale as well as semi-structured interviews were used for the data collection. Analysis of the data was mainly performed by inferential statistical mainly non-parametric methods and by a phenomenographic approach. The result showed that women with breast cancer in stages I to II could be followed up by a specialist nurse leading to high patient satisfaction and good medical safety. Women tend to vary in their appreciation of different aspects of the follow-up; some need routine while others require accessibility, continuity, confidence and security were demanded as self-care education and individualised information. A multidisciplinary education programme based on patients' needs led to a similar level of well-being, self-care and coping ability as that resulting from traditional physician follow-up and thus can be considered as a viable alternative.

The women value the nurses professional knowledge and skills. Accessibility and early assessment by healthcare professionals or an oncology nurse are essential in a system without routine follow-up. There are good reasons for reviewing and changing the design of the traditional follow-up system in order to ensure the most effective and well-functioning system possible, to better meet the needs of women with breast cancer and to involve them in decision making concerning follow-up.

December 2004

Search for susceptibility loci and candidate genes for breast cancer

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Breast cancer is the most frequent cancer among women in the western world. Family history is a well-established and important risk factor for breast cancer. Both a population-based twin study and a study based on a large family cancer database have suggested that hereditary factors account for about a quarter of breast cancers. The well-established high penetrance genes, e.g. BRCA1 and BRCA2, only account for less than 5% of all breast cancer cases. In attempts to identify further genetic factors, two common approaches have been used. One is linkage analysis, which is used to pinpoint the putative moderate/high susceptibility loci. The other is an association study, which is thought to be more powerful in the search for low-penetrance alleles in candidate genes.

Here, we explored the susceptibility loci in chromosomes 1, 13, 16, and 17 in Swedish monozygotic twins, concordant for breast cancer, using loss of heterozygosity (LOH) analysis. We also carried out association studies to investigate the contribution of functional polymorphisms in putative breast cancer susceptibility genes to the risk of breast cancer, using both unselected and familial breast cancer cases. The use of familial cases can significantly increase the power of the association studies. The genes selected were the sex hormone binding globulin (SHBG) gene, genes involved in transforming growth factor β (TGFβ) signaling pathway, and the vascular endothelial growth factor (VEGF) gene.

In the LOH study, our hypothesis was that loss of the same allele at a specific genomic region in both of the twins might suggest a tumor suppressor gene that confers a strong predisposition to breast cancer. From the analysis, we found that 16qtel and 17p13 were the two main candidate regions in the twins. 16q22.1, 17q21 and 13q14 may also be involved in a subset of the twins. Chromosome 1 did not seem to carry any important tumor suppressor gene in our sample set.

Estradiol is one of the strongest risk factors for breast cancer. SHBG is a plasma carrier of estradiol and it is involved in regulation of the bioavailability of estradiol to target cells. We analyzed three functional coding-region polymorphisms in the SHBG gene. One of them appeared to have a small protective effect on breast cancer risk, both in the unselected and the high risk breast cancer populations. The effect became significant when all breast cancer cases were compared to all of the controls.

Five polymorphisms in the TGFβ1 gene, one polymorphism in the TGFβRI gene and two polymorphisms in the TGFβRII gene were analyzed. In normal human breast epithelial cells and during the early stages of breast cancer development, TGFβ1 acts as a growth inhibitor but with progression as a tumor promoter. Genotype and haplotype analyses were carried out on the polymorphisms in the TGFβ1 gene, and a genotype combination analysis on the polymorphisms in the TGFβ1 and its receptor genes. Only carriers of the rare 6A/6A genotype in the TGFβR1 gene were suggested to be at an increased risk of breast cancer.

Formation of new blood vessels (angiogenesis) is an important step during the development of cancer. VEGF is a major mediator of breast cancer angiogenesis. Altogether four polymorphisms in the VEGF gene were studied in both familial and unselected breast cancer cases, together with corresponding controls. None of the polymorphisms, nor any haplotype alone, were significantly associated with either familial or unselected breast cancer. However, when we investigated the VEGF genotypes in relation to the clinical characteristics in the unselected breast cancers, some of the genotypes and haplotypes showed significant association with altered tumor aggressiveness.

In summary, we found that 16qtel and 17p13 are the two main candidate breast tumor suppressor gene regions among the chromosomes studied in Swedish monozygotic twins. Some of the genetic polymorphisms in the genes that were studied may influence the risk of breast cancer. Furthermore, some genotypes and haplotypes appear to affect the development of breast tumors.

December 2004

Modulation of tumor sensitivity to effector mechanisms of cytotoxic lymphocytes

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Today, ample evidence demonstrates a clear role for the immune system in the battle against cancer. However, the relatively high rate of mutation and proliferation of tumor cells, in combination with the selective pressure exerted by the immune system, can potentially lead to the generation of genetically altered tumor cells, which are able to evade recognition by the immune system and continue to grow and form tumors. Increased knowledge of the mechanisms allowing tumors to escape from the immune system is of great importance in facilitating the design of effective immunotherapeutic regimens against cancer. The work described in this thesis was aimed at identifying new mechanisms of tumor escape as well as possible ways to counteract them.

We have identified TNF-α as a potent modulator of MHC class I antigen presentation in tumors. TNF-α-treatment led to enhanced expression of several molecules in the MHC class I antigen processing and presentation pathway, including the IFN-inducible subunits of the proteasome, LMP2, LMP7 and MECL-1, the transporters associated with antigen presentation (TAP) and MHC class I heavy chain. These changes resulted in increased stability of surface MHC class I complexes, presumably due to an increased supply of peptides suitable for binding to MHC class I molecules, and enhanced susceptibility of TNF-α-treated tumors to antigen-specific lysis by cytotoxic T-lymphocytes (CTLs). Our results suggest a role for TNF-α as a potent immunomodulator in IFN-γ unresponsive tumors.

Investigating the possible effects of cytokines on the sensitivity of tumor cells to different CTL effector mechanisms, we found that IFN-γ protects uveal melanoma cells from CTL-mediated lysis. We also demonstrated that despite potent upregulation of antigen presentation in uveal melanoma cells, IFN-γ-treated tumor cells were less sensitive to lysis by CTL. Granzyme B is an apoptosis-inducing effector molecule released by CTLs upon triggering of the T-cell receptor. IFN-γ-treated uveal melanoma cells bound less granzyme B than their untreated, or TNF-α-treated, counterparts. Cleavage of the granzyme B substrate Bid was reduced in uveal melanoma cells following treatment with IFN-γ. This correlated with a reduced expression of the cation-independent mannose-6-phosphate receptor (CI-MPR), a receptor for granzyme B, and decreased CTL-lysis of IFN-treated uveal melanoma cells. In another study, we examined the regulatory role of IFN-γ on the sensitivity of uveal melanoma cells to the lytic activity of perforin, another major constituent of cytolytic granules. We demonstrated that IFN-γ induces resistance of uveal melanoma cells to plasma membrane lysis by perforin. This was not a result of proteolytic inactivation of perforin by either cathepsin B, known to protect CTL from perforin-mediated suicide, or other proteases. Protection from perforin lysis correlated with IFN-γ-induced growth arrest in the G_1-phase of the cell cycle, and reduced binding of perforin to IFN-γ-treated OCM1 cells. In light of the current data, we propose a mechanism where IFN-γ-induced growth arrest leading to structural changes in the plasma membrane results in decreased perforin binding capacity of the tumor cell and protection from perforin. Our results demonstrate that, in response to IFN-γ, tumors can escape the immune system through the active acquisition of a CTL-resistant phenotype, characterized by impaired sensitivity to granule-mediated killing.

The second major effector mechanism employed by CTL is the engagement of death receptors expressed on target cells. The production of soluble Fas ligand (sFasL) completely protected uveal melanoma cells from killing via Fas. Inhibition of metalloproteases on the surface of tumor cells prevented shedding of FasL and rendered uveal melanoma cells sensitive to Fas-meditated lysis by CTL. The protective effect of FasL was not due to tumor counter-attack or reduced lytic potential of CTL, but transfer of sFasL-containing culture supernatant protected normally Fas-sensitive cells from killing induced both by FasL-expressing lymphocytes and a agonistic antibody to Fas. We speculated that soluble FasL bind to Fas receptors expressed on tumor cells, thereby preventing their activation by Fas-inducing effector molecules. Our findings demonstrate the existence of a novel mechanism of tumor escape from death receptor-mediated killing by cytotoxic lymphocytes, and point to a new rationale for the use of metalloprotease inhibitors as cancer therapeutic agents.

December 2004

The identification and characterisation of LRIG gene family and its expression in astrocytic tumours

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Gliomas are the most common primary brain tumours, and their capacity to invade surrounding normal brain prevents complete removal of the tumour. Malignant glioma has still a poor prognosis. However, with the rapid development of molecular biology our understanding about glioma has increased dramatically. Among known growth factors, EGF and its receptor are frequently amplified and over expressed in malignant glioma. Therefore, it is of interest to find approaches to hamper the activity of EGF/EGFR. The aim of this thesis was to identify and characterize human analogues to a recently identified gene in Drosophilia, kekkon-1, which negatively regulates the activity of Drosophilia EGF receptor.

In the first part, we set up a quantitative real-time RT-PCR assay, which showed good linearity, reproducibility and uniformity. We analyzed the expression of the most commonly used

reference genes, and showed that 18S was the most reliable endogenous reference gene in this study.

In the second part, we cloned, identified, and sequenced a gene family, which we named leucine-rich repeats and immunoglobulin–like domains family (LRIG). The LRIG gene family had three vertebrate paralogs and one homolog in ascidiacea. The proteins encoded by human LRIG genes shared an overall structure with a signal peptide, 15 tandems leucine-rich repeats with N- and C-terminal flanking regions followed by 3 immunoglobulin-like domains, a transmembrane domain, and a cytoplasmic tail. Northern blot showed the mRNA sizes to be 5.5 kb for LRIG1, 4.8 kb for LRIG2, and 5.1 kb for LRIG3. LRIG1-3 mRNAs were detected in all human and mouse tissues analyzed, however, at various levels. FISH and BLAST analysis showed that LRIG1 was located at 3p14, LRIG2 at 1q13, and LRIG3 at 12q13. LRIG1 was shown to be down-regulated in several cancer cell lines and proposed to be a tumour suppressor gene.

In the third part, we analysed the expression of LRIG gene family in human astrocytic tumours. LRIG1-3 mRNAs were detected in all human glioma cell lines, in primary tumour tissues and control-matched normal brain tissues, at various levels. Subcellular localizations of LRIG1-GFP fusion proteins were visualized in nuclear, perinuclear, and cytoplasmic compartment. According to the predicted protein sequences, short peptides were synthesized and used to raise antibodies in rabbits. The antibodies were used for immunohistochemical analysis of LRIG1-3 in 404 human astrocytic tumours in a tissue micro array. The pattern of immunoreactivity of LRIG1-3 was heterogeneous with staining in nuclear, perinuclear and cytoplasmic compartment of positive tumour cells. Perinuclear staining of LRIG1-3 displayed a significant inverse correlation with WHO grade and especially positive LRIG3 perinuclear and cytoplasmic staining correlated with a low proliferation index. The LRIGs correlated with survival, and LRIG3 perinuclear staining was in addition to tumour grade an independent prognostic factor. The results suggest that LRIGs may play a role in normal tissue, and may be of importance in the pathogenesis and prognosis of tumours. The exact function of LRIG1-3 remains to be established.

December 2004

Parents with cancer – The experiences of family responsibility

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The main aim of this study was to gain an understanding of how women and men with cancer illnesses, looking after children at home, experience and manage their life situation, with focus on the responsibility as parent. An additional aim was to identify the parents' need for support, in order to develop support activities for them from a family nursing perspective. The perspective of symbolic interactionism influenced the qualitative studies in the thesis. Concepts used in the thesis were *transition,* identified as a core concept in nursing, and *mothering* and *ethics of care* as sensitizing concepts. The emphasis was on a conceptual framework in nursing, encompassing the main components of the theory of transition. The analysis methods used were grounded theory, interpretive description and secondary analysis. Data was collected from open interviews with women and men with cancer who had children living at home. The participants in study I were nine women with breast cancer, in study II, eight men with cancer in the circulatory system and in study III, ten women with cancer in blood systems and a focus group interview. Study IV *involved* the same participants as study I and III. The results of study I illustrate the main theme of how the lives of these women had changed: transforming the exhausting-to-energising process of being a good parent in the face of cancer. All of the women expressed the desire to be a good mother. In study II, a central theme was generated – change in self-image as a man and as a parent. The men's self-image changed as well as their function as parents. In study III the core concept identified was the experience of dealing with the moral responsibility of being a mother with cancer. The findings were presented as a life story where the women's experiences were weaved together. In study IV, the three phases in the transition process were used and a main theme was constructed that integrated these phases: 'the desire to manage one's responsibility as a parent' within the context of mothering. All of the women included in this study expressed the need for professional support to help them to endure treatment procedures as well as to sustain their moral responsibility as good mothers. A model for professional moral support is suggested, based on these findings. The research implications are that the concept of moral support has been identified and can be incorporated into a theoretical model in order to further generate a hypothesis with the aim of creating a useful clinical intervention model. The clinical implications of this are to use and evaluate the tentative model for moral support in clinical practice in patient encounters with the family and in training health care staff.

December 2004

Intraperitoneal 5-fluorouracil treatment of cancer – Clinical and experimental studies

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Pancreas cancer is a most aggressive malignancy. More than 80% of patients diagnosed with pancreas cancer, exhibit such advanced disease, that curative surgery is impossible. Systemic chemotherapy prolongs survival to 5-9 months. High concentrations of chemotherapeutic agents in the abdominal cavity and in the lymphatics draining the area is achieved by intraperitoneal administration. Vasopressin decreases splanchnic blood flow, reducing the intraperitoneal uptake of drugs, thus raising the local and lymphatic dose intensity. The aim of the study was to investigate the feasibility and tumour response of intraperitoneal 5-fluorouracil (5-FU) treatment in non-resectable pancreas cancer, using vasopressin to improve the pharmacokinetic profile. Further, to study the effect of vasopressin on peritoneal blood flow, altered by intraperitoneal 5-FU or the presence of peritoneal carcinomatosis.

In the animal experiments, the ^{133}Xe-clearance technique and as a comparison Laser doppler flow, were used to identify changes of peritoneal blood flow caused by vasopressin. The ^{133}Xe-clearance technique was used to identify changes of peritoneal blood flow caused by vasopressin in animals with peritoneal carcinomatosis or animals given intraperitoneal 5-FU. In the clinical studies, 68 (39 women/29 men) patients, with a non-resectable ductal pancreas cancer and a Karnofsky Index $=70$ were included. Patients were treated with 750–1500 mg/m^2 5-FU intraperitoneally through a Port-a-cath and Leucovorin 100 mg/m^2 intravenously on two consecutive days every 21 days until progression. Seventeen patients, receiving 750 mg/m^2 5-FU, were

given concomitant vasopressin 0.1 IU/min during 180 minutes, alternatively day 1 or 2.

In the animal experiments, vasopressin 0.07 IU/kg/min significantly reduced the ^{133}Xe-clearance. Intraperitoneal 5-FU decreased the basal peritoneal blood flow and abrogated the vasopressin effect for 1–2 days. The presence of peritoneal carcinomatosis did not influence the basal peritoneal blood flow, nor the reduction of peritoneal blood flow caused by vasopressin. In the clinical studies, the treatment with intraperitoneal 5-FU was well tolerated, with no WHO grade 3 or 4 toxicity with doses up to 1250 mg/m^2. Thirty patients achieved at least stable disease at three months. The median survival time was 8.0 (range 0.8–54.1) months. There was a significant reduction of 5-FU Cmax on day 2, but no significant reduction of AUC, when vasopressin was given.

Peritoneal blood flow changes caused by vasopressin can be estimated with the ^{133}Xe-clearance technique. Intraperitoneal 5-FU but not peritoneal carcinomatosis decreases the vasopressin induced ^{133}Xe-clearance reduction, 1–2 days after administration. In patients with non-resectable pancreas cancer, intraperitoneal 5-FU up to 1250 mg/m^2 for two days every third week can be given without WHO grade 3 and 4 toxicity. The treatment is well tolerated with few and minor side effects. Tumour responses were observed. Addition of vasopressin does not significantly enhance the pharmacokinetics of intraperitoneal 5-flurorouracil, but adds toxicity.

December 2004

Professional caregivers' experiences of caring for women with breast cancer on a surgical ward

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The overall aim of the thesis was to describe caregivers' experiences of caring for women with breast cancer on a surgical ward. The study was based on interviews with narrative parts and tape-recorded clinical supervision sessions. The interviews and clinical supervision sessions were transcribed verbatim, and analysed by content analysis.

Nurses (n = 10) described life for women with breast cancer as either having freedom or not having freedom, with both physical and existential suffering. Dying occurred either naturally in patients' own home or unnaturally in hospital. The nurses felt that it is possible to alleviate suffering during dying through providing adequate pain relief but also, through listening, providing information and changing the caring atmosphere.

Breast cancer as an illness was described from a dark point of view by caregivers (n = 37). The descriptions focused on loss of breasts and control, progression of the illness and annihilation. The illness seemed, in the caregivers' mind, to often end with a painful death. Caregivers who had the opportunity to follow the total care process described a lighter viewpoint.

According to nurses (= 31) the most important needs among women, their relatives and nurses themselves were the needs to talk and receive information. There was a discrepancy between what was described as important needs and the descriptions of how these needs were provided for. Nurses, whose own needs for support were sometimes unsatisfactorily met, seemed almost to be unaware of the needs among women and their relatives.

In the clinical supervision sessions caregivers reflected on difficult care situations related to women's, relatives', and most often caregivers' feelings (n = 38). The care situations were described as evoking feelings of discomfort, powerlessness and reduced self-esteem. These feelings were described by caregivers as arising in connection with caring for especially women with advanced breast cancer in a changing organisation.

Caregivers' descriptions of caring for women with breast cancer show a lot of negative experiences of powerlessness and frustration. They met women and their relatives who suffered in various ways and had considerable need for support. Caregivers often found themselves unable to meet these needs due to organisational obstacles e.g. lack of time and lack of knowledge about other caregivers' responsibility in the care.

December 2004

Stress and coping in parents of children with cancer

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The general aim of this thesis was to investigate disease-related stress and ways of coping in parents whose children were in active treatment for cancer, or had completed successful cancer treatment. Specifically, the research included examinations of: disease-related stress at various points in time after the child's diagnosis; strain and traumatic stress during and after the child's treatment; the relation of certain demographic and disease-related variables to parental stress; the use of various coping strategies, and the co-variation of coping strategies and level of emotional distress; and the relationships between perceived social support, support-seeking coping, and emotional distress.

The four sub-studies of the thesis involved cross-sectional samples including 265, 413, 395, and 184 parents, respectively. Parents were recruited at Astrid Lindgren Children's Hospital, Stockholm, and at Linköping University Hospital. Both mothers and fathers were invited. The time elapsed since disclosure of the child's diagnosis varied from one week to fourteen years. All four studies were based on quantitative data, collected through self-report inventories. Fourteen various aspects of disease-related stress, and seven types of coping strategies were examined.

Findings indicate that high levels of disease-related distress are particularly frequent among parents during the first period after the diagnosis. However, most aspects of disease-related strain were reported by parents later in time as well. Indeed, years after the diagnosis parents were more anxious than parents of healthy children. Furthermore, although particularly the treatment phase appeared to involve events that affect parents' experience of control, as well as elicit traumatic stress reactions, most of the assessed aspects of stress seemed to occur among parents of children off treatment as well.

Positive perceptions of social support, and a coping style that included problem-focusing appeared to make parents less affected by strain. In contrast, the reliance on a coping style including a passive reaction pattern was associated with higher levels of anxiety and depression. An avoidant coping style was also associated with more distress. However, immediately after a child's cancer diagnosis, the distress seemed to be high regardless of whether parents relied on avoidant coping or not.

Findings indicate that parents with lower education and non-Swedish origin may be less resilient to traumatic stress after end of treatment, than parents with higher education levels and a native Swedish background. In contrast, a good prognosis did not seem to make parents less vulnerable to distress than a worse prognosis

or a relapse in the child. Moreover, cancer in a child appeared to affect mothers and fathers similarly.

In conclusion, the data suggest that several aspects of disease-related strain are relevant in various patterns to parents during the child's treatment as well as when treatment is completed, and that such strain can appear at any point in time after a child's cancer diagnosis. Factors other than the passing of time and the termination of treatment account for the majority of variation in parental stress.

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Tumor lipid status and the response to therapy in neuroblastoma with emphasis on treatment monitoring by proton magnetic resonance spectroscopy

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In this thesis, the aberrant lipid and phospholipid metabolism of neuroblastoma was exploited for tumor monitoring and as target of experimental therapy. Neuroblastoma is the most common and deadly solid tumor of childhood. Since neuroblastoma cells are highly proliferative, aberrantly regulated metabolic pathways would be expected. Lipids and phospholipids were considered relevant to monitor and target, since these biomolecules are required for membrane synthesis and signal transduction.

Human neuroblastoma xenografts in athymic rats were analyzed in vivo by proton magnetic resonance spectroscopy (^{1}H-MRS), a clinically available method previously found useful for assessment of tumor (phospho)lipid metabolism.

A significant association between the in vivo content of choline-containing species (total choline), MRS-detectable lipids, and neuroblastoma tissue viability was demonstrated. The in vivo lipid/choline ratio was significantly inversely correlated with the viable tumor fraction. Histological response of neuroblastoma xenografts to the angiogenesis inhibitor TNP-470 was associated with an enhanced lipid/choline ratio.

Neuroblastoma growth arrest induced by serum starvation in vitro was associated with a decreased intensity of most ^{1}H-MRS-detectable metabolites, in particular total choline. The mobile lipid/choline ^{1}H-MRS ratio was validated in vitro and in rats in vivo as an accurate predictor of neuroblastoma chemotherapy response. The lipid/choline ratio increase was observed in drug sensitive xenografts in vivo within two to three days of treatment with irinotecan, predicting and preceding tumor regression. No corresponding metabolic alterations were detected in saline treated tumors or in multidrug resistant irinotecan-treated tumors.

Neuroblastoma tissue from patients was analyzed for the expression of cyclooxygenase-2 (COX-2), an enzyme that catalyzes prostaglandin formation from the *n*-6 polyunsaturated fatty acid arachidonic acid, abundant in cell membranes. COX-2 was expressed in neuroblastoma tumors and cell lines, but not in non-transformed childhood adrenal medulla. Treatment with COX-inhibiting non-steroidal anti-inflammatory drugs (NSAIDs) induced apoptosis of neuroblastoma cells, a process associated with mitochondrial depolarization, activation of caspase-9 and caspase-3, and an enhanced ^{1}H-MRS lipid/choline ratio. The NSAIDs diclofenac and celecoxib induced caspase-3 activation in vivo, and significantly inhibited neuroblastoma xenograft growth.

Supplementing cell lines with the polyunsaturated fatty acid docosahexaenoic acid (DHA, *n*-3), as opposed to the monounsaturated oleic acid (OA, *n*-9), induced neuroblastoma cell death by mechanisms involving enhanced susceptibility to oxidative stress. DHA, but not OA, caused mitochondrial membrane depolarization in a cyclosporine-sensitive manner, and significantly enhanced neuroblastoma response to treatment with chemotherapy, NSAIDs, or a clinically relevant concentration of arsenic trioxide. Fibroblasts were not substantially affected by DHA.

In conclusion, based on aberrant tumor lipid metabolism, MRS-based surrogate markers of neuroblastoma viability and treatment response can be monitored non-invasively in animal models. Targeting of neuroblastoma fatty acid homeostasis by NSAIDs or DHA is feasible in preclinical neuroblastoma models, and warrants clinical testing as potential novel modes of therapy with improved neuroblastoma selectivity.

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Mobile phone use and risk of intracranial tumors

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Mobile phones are today an integral part of many people's lives in western societies. The phones emit radiofrequency radiation when being used. Concerns have been raised about possible effects that the exposure may have on the health of the mobile phone user. If radiofrequency radiation has a carcinogenic potential the exposure might pose an important public health problem. The overall aim of this thesis was to study the association between radiofrequency exposure from mobile phone use and risk of intracranial tumors.

The aim was to describe the incidence trends of intracerebral tumors during a period with introduction of new diagnostic procedures and increasing prevalence of mobile phone users. Information about adult primary intracerebral tumors was obtained from national cancer registries in four Nordic countries for the years 1969-1998. Estimates of person-years at risk were calculated from the information obtained from national population registries. Annual age standardized incidence was calculated and time trends were analyzed. The result showed an increase in the incidence that was confined to the late 1970s and early 1980s and coincided with the introduction of improved diagnostic methods such as computerized tomography. After 1983, and during the period with increasing prevalence of mobile phone users, the incidence has remained relatively stable for both men and women. The purpose of the distribution of power levels from mobile phones in four geographical areas with different population density was analyzed to see whether region was a significant determinant of exposure. The output power for all mobile phone calls managed by the GSM operator Telia Mobile was recorded during one week in four defined areas in Sweden. In the rural area, the highest power level was used by mobile phones about 50% of the time, while the lowest power was used only 3% of the time. The corresponding numbers for the city area were approximately 25% in the highest and 22% in the lowest. A case-control study on intracranial tumors and radiofrequency exposure from mobile phone use was presented. It includes all residents aged 20 to 69 years in geographical areas covered by four regional cancer registries in Sweden. Eligible cases were all subjects diagnosed with acoustic neuroma, glioma, meningioma, and parotid gland tumors. Cases were identified continuously during the study

period at the clinics at all hospitals within the study area. Controls were randomly selected from the study base. Detailed information about mobile phone use and other environmental exposures was collected by personal interviews. The hypothesis that radiofrequency exposure from mobile phones increases the risk of acoustic neuroma was tested as well as the hypothesis that radiofrequency exposure from mobile phones increases the risk of glioma and meningioma. The results displayed that ten or more years of mobile phone use increases the risk of acoustic neuroma and that the risk increase was confined to the side of the head where the phone was usually held. No indications of an increased risk for less than 10 years of mobile phone use were found. No increased risk was found for glioma or meningioma related to mobile phone use.

The results in this thesis are in accordance with results from most previous studies. The results do not support the hypothesis that radiofrequency exposure from mobile phone use increases the risk of glioma or meningioma. The results do not support the hypothesis that short-term mobile phone use increases the risk of acoustic neuroma, but the results indicate that long-term use increases the risk of acoustic neuroma. This result needs to be confirmed in other studies before firm conclusion could be drawn.

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Leukaemia chemotherapy-Experimental studies on pharmacological optimisation

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Our main goal has been to identify new means to improve chemotherapy of acute leukaemia:

By using low-density lipoprotein (LDL) as a drug carrier to increase the selectivity of antileukemic drugs, based on high LDL uptake in acute myeloid leukaemia (AML) cells. Our first concern was to investigate the importance of chemical structures to obtain a stable anchorage of the drug into LDL. The only stable complex was obtained when incorporating cholesteryl-linoleat in LDL as shown by dialysis and autoradiography data. With N-trifluoroacetyl-adriamycin-14-valerat-LDL (AD32-LDL) the drug leaked slowly into the plasma. In AML patients a rapid plasma dissociation of AD-32-LDL was observed, illustrating a much higher in vivo instability of this complex. We thereafter synthesised five lipophilic derivatives of daunorubicin (DNR) for incorporation into LDL. Three incorporated successfully into LDL: 2 benzyloxy and the isonicotinoyl derivatives. In vitro these complexes were more cytotoxic towards a LDL receptor positive cell line than to LDL receptor negative cells, but non-specific cytotoxicity was quite high and was explained by slow dissociation of the drug-LDL complexes in plasma. These results underline the difficulty in obtaining a stable LDL complex. Finally we studied the cytotoxicity of WB4291, a lipophilic alkylating agent, after incorporation in LDL or lipid microemulsions towards sensitive and resistant myeloid cell lines. The complexes exerted a better activity than melphalan and DNR towards all the resistant sublines expressing Pgp, K562/Vcr and/Dnr.

By studying the relation between DNR concentration and apoptosis induction in leukemic cells. We studied the time course of induction of apoptosis by DNR in HL60 and K562 cells and in isolated leukemic cells from patients with AML, after a pulse incubation with increasing drug concentrations. Caspase-3-like activity correlated positively with DNR concentrations, appearing faster at high DNR concentrations in all the cells. DNA fragmentation occured in two steps, an intranucleosomal cleavage producing high MW DNA fragments, followed by an internucleosomal cleavage and the apparition of small fragments observed in a typical DNA ladder. The high MW fragments were observed in all the cells with the exception of K562 cells. DNA fragmentation was faster at high DNR concentrations in all the cells except K562 cells. In leukemic cells from patients with AML, the time course of DNA fragmentation at 0.25 μg/ml showed large interindividual variations in in vitro chemosensitivity that could reflect variations in in vivo sensitivity. The results support the concept of dose intensification in induction therapy with DNR.

By studying of the impact of cell density on drug cytotoxicity in order to optimise individual treatment. White blood cell count (WBC) is generally accepted as a significant prognostic risk factor in acute leukemia outcome and shows a marked variation at diagnosis. Actually the dose regimen currently used ignores the size of the tumor burden and the standardization of the dose is generally based only on body surface area. In this study we investigated the effect of cell density on the cytotoxic activity of DNR and Ara-C in HL60 cells and in leukemic cells isolated from patients with AML. We showed that their cytotoxicity decreased with cell density and that apoptosis induction in isolated leukemic cells by DNR was reduced at higher cell density. This correlated with the marked reduction of DNR and AraC uptake in HL60 cells at high cell density. We hypothesised that a high WBC will lower the plasma concentration through a high uptake in the tumor cells and in this way decrease the drug concentration in leukemic blasts. In patients with high WBC, a dose increase and an optimal administration schedule should be evaluated for treatment with DNR and/or AraC.

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Analysis and manipulation of autoreactive and tumor-specific T cell responses

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The focus of this work has been on the analysis and manipulation of T cell responses. One emphasis has been on T cells involved in autoimmune diabetes and another on T cells in tumor immunity. Primary activation of T cells as well as the role of the proteasome in T cell responses has been overlapping themes.

Non obese diabetic (NOD) mice represent a mouse model for human type 1 diabetes. Diabetes at NOD mice starts at around two weeks of age, when naïve autoreactive T cells encounter β cell-derived antigen, in the pancreatic lymph nodes (PLN). Activated T cells proliferate at this site and subsequently leave the PLN and infiltrate the pancreas. Lymphocytes first appear in the pancreas at 3 weeks of age and diabetes occurs around 12 weeks of age. In an attempt to identify disease-initiating T cells, we studied the length of the CDR3 region on T cells isolated from the PLN of very young NOD mice. Immunoscope analysis of lymph node RNA from different time points (10, 14, 18 and 22 days of age), revealed subtle and transient oligoclonal expansion of TCRVβ5.1-Jβ1.5, TCRVβ5.1-Jβ1.3, TCRVβ5.2-Jβ1.5 and TCRVβ5.2-Jβ1.6 in the PLN in comparison to the inguinal lymph node (ILN). Targeting those specific TCR species may offer novel treatment strategies in autoimmune diabetes.

The subtleness of the initiating events in the PLN suggested that variation in activation thresholds could affect rate-limiting steps in order for T cell activation to proceed. One such threshold may be stimulation needed for individual T cells to initiate IFN-γ secretion. We found that naïve $CD8^+$ T cells are geared towards IFN-γ production when stimulated with low amounts of peptide, suggesting that they may play a role in early phases of immune responses in peripheral lymphoid organs.

Another important focus of this thesis has been attempts to manipulate T cell responses using, proteasome inhibitors. Proteasome inhibitors affect peptide presentation but also modulate cytokine production, proliferation and survival of T cells.

Applying proteasome inhibitor MG132 in a diabetes transfer model reduced diabetes with 76% in inhibitor treated mice compared with control recipients. The calpain inhibitor z-Leu-Leu-H was without protective effect suggesting that MG132 acted via inhibition of the proteasome. Leukocyte infiltration of the pancreatic islets was not dramatically altered. However, our analysis revealed a decrease in proliferation of the transferred T cells in the PLN of the recipient mice, suggesting that the link between PLN proliferation and islet infiltration may reflect qualitative rather than quantitative effects.

The use of proteasome inhibitors in a tumor system revealed a role of the proteasome in the production of certain tumor antigens. When tumor cells were adapted to grow in the presence of proteasome inhibitors they became more efficient in avoiding immunological reaction and formed more tumors in vivo. This phenotype might be preferentially selected in tumors to allow immune escape.

Together, the studies presented in this thesis contribute to the understanding of T cell activation in autoimmune diabetes and cancer and propose different possibilities of modulation activation, proliferation and effector function of T cells.

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Colon cancer Disease related proteins in tumor tissue and serum

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Despite the progress in surgical techniques and adjuvant therapies the mortality of around 60% within the group of colon cancer patients did not decrease significantly over the last decades. This determines a growing demand for biomarkers for early detection, prognosis and risk assessment in colorectal malignancies. Desirable criteria for such a biomarker test are minimal burden and maximum safety for the patient, cost efficiency and broad acceptance to reach a high compliance of the patients.

More than 300 colon tissue samples and 1000 sera were obtained from CRC and FAP patients at the Lübeck and Düsseldorf University Hospitals in Germany. To identify the consequences of genetic aberrations on protein expression level, samples from patients with advanced sporadic colon cancers in which the corresponding mucosa, adenoma, carcinoma and liver metastasis were available, were analyzed by 2-D gel electrophoresis and mass spectrometry. A total of 46 proteins were found to be upregulated during the progression of sporadic cancer, and 26 were downregulated. Several of the identified polypeptides correlate with proteins regulating specific cell functions (cell cycle, cytoskeleton, metabolic pathways). In a further study we used a 2-DE based proteomic approach to compare the expression pattern of normal colonic mucosa vs. mucosa gained from FAP patients, polyps vs. FAP polyps and sporadic vs. FAP associated cancer, respectively. A total of 47 proteins were always present in FAP mucosa and absent in the normal mucosa. Based on a total of 37 proteins FAP polyps and sporadic polyps could be distinctly separated. In addition, the absence/presence pattern of 66 spots allowed to distinguish FAP cancers from sporadic cancers. These data suggest that proteome analysis makes it possible to diagnose FAP already on macroscopically normal appearing colonic mucosa. By means of SELDI array based investigations we unrevealed 16 serum proteins that were able to classify 98% of all test set samples correctly. Our SELDI results show that serum marker protein profiling enables to diagnose and discern malignant colon cancer patients from healthy individuals. Although these markers need validation before they can be used in clinics they have potential for the design of a marker panel for objective diagnosis and therapeutic strategies for colorectal cancer and metastasis.

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Molecular genetic aspects of colorectal cancer development

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Colorectal cancer (CRC) is one of the most common cancer diseases in the world after lung and female breast cancer and approximately 945 000 new cases are diagnosed every year. CRC is caused by genetic alterations in the DNA, which results in cell cycle acceleration, escape from apoptosis, senescence, angiogenesis, invasion and metastasis. In this thesis, we have investigated molecular genetic alterations for the development of CRC and focused on the MAPK pathway, *HIF-1α* and *NOS2* genes.

Alterations in the MAPK pathway have been found in several different cancer forms, including CRC. In the present study, we found somatic mutations in the MAPK pathway in 50% of the CRCs; 40% of the tumors carried mutations in the *KRAS* gene and 10% carried *BRAF* mutations. No genetic alterations were found in the *ARAF* or *RAF-1* genes. *BRAF* gene mutations were present only in exon 15 and were associated with microsatellite instability. Three mutation types were identified; V599E, D593G and K600N, whereof the latter has not previously been described.

The hypoxia inducible factor (HIF)-1α protein is involved in the oxygen sensing mechanism and several tumor types show HIF-1α overexpression due to hypoxia. At normoxia, HIF-1α is degraded by interaction with the von Hippel-Lindau (VHL) tumor suppressor protein followed by an ubiquitin-proteasome dependent degradation mechanism, which prevents HIF-1α from nuclear translocation and transcription of downstream target genes. Fifteen percent of CRC patients and normal healthy population was found to carry the P582S polymorphism in the *HIF-1α* gene, which previously has been associated to higher transactivating capacity. In the present study, the polymorphism was associated to ulcerative tumor development. In addition, loss of heterozygosity of the wild type P582 allele in heterozygotes may contribute to the development of ulcerative CRCs. However, the overall mechanism for ulcerative tumor development is still unclear.

Nitric oxide (NO) is involved in several physiological processes, such as apoptosis, neurotransmission, angiogenesis and immune defence and is produced by three nitric oxide synthases; NOS1-3. In the present study, NOS2 upregulation was identified in CRCs compared to normal intestinal mucosa. Moreover, the contribution of NOS2 in CRC development was investigated in $APC^{Min/+}$ and $APC^{Min/+}$ $NOS2^{-/-}$ mice. The $APC^{Min/+}$ $NOS2^{-/-}$ mice developed a higher polyp frequency compared to $APC^{Min/+}$ mice, indicating a protective role for the presence of NOS2 in intestinal cancer development. The elevated polyp formation in the $APC^{Min/+}$ $NOS2^{-/-}$ mice was independent of the expression of Notch-1 and p21. We also investigated whether polymorphisms in the NOS2 promoter affected the onset of CRC, but no differences in allele or genotype frequencies were observed in normal healthy population compared to CRC patients

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Functional characterization of the liposarcoma-associated fusion oncogene *FUS-DDIT3*

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Fusion genes represent a growing class of translocation-derived potent oncogenes that frequently show tumor type-specific expression. We have studied the myxoid/round cell liposarcoma (MLS/RCLS)-specific *FUS-DDIT3* fusion, with the aim of functionally characterizing this fusion oncogene in sarcoma development. *FUS-DDIT3* is the result of a chromosomal translocation t(12;16)(q13;p11) which fuses the 5′ end of *FUS (TLS)* with the entire *DDIT3 (CHOP)*. As a result, the fusion gene is transcriptionally controlled by the constitutively active *FUS* promoter. The causative role of the *FUS-DDIT3* fusion in initiation of MLS/RCLS has been demonstrated in transgenic mice. Ectopic expression of DDIT3 and the FUS-DDIT3 proteins have been shown to counteract differentiation and abrogate adipocyte development. This results in partially committed pre-adipocytes, a cell population with the potential to progress towards liposarcoma development.

We have developed an experimental system, consisting of genetically modified human fibrosarcoma HT1080 cells stably expressing FUS-DDIT3, C-terminally truncated FUS or DDIT3, coupled with the Green Fluorescent Protein (GFP). By using this system we have been able to study the localization of these proteins, their interaction partners and the different gene expression profiles induced by their expression. We have found that the FUS-DDIT3-GFP fusion protein localizes to well-defined nuclear structures. This enabled us to study the interaction between FUS-DDIT3 and other nuclear proteins. We have found that FUS-DDIT3 associates with the splicing machinery and proposed a model in which this fusion gene disturbs the normal splicing process, an idea later confirmed by others in splicing assays.

By microarray analysis, we identified the *Il.6* and *Il.8* genes as FUS-DDIT3 targets and demonstrated for the first time that DDIT3 and FUS-DDIT3 initiate opposing transcriptional regulation of the *Il.8* gene. IL6 is a multi-functional cytokine that has been shown to act as an autocrine growth factor in human prostate cancer cells and the existence of an IL6 autocrine loop has been implicated in the oncogenesis of multiple myeloma. In addition, it has been shown that IL6 plays a pivotal role for proliferation and invasion of malignant fibrous histiocytoma. It is possible that aberrant *Il.6* expression has similar functions in MLS/RCLS.

Immuno-histochemical analysis involving 5 primary non-irradiated and 12 secondary and/or irradiated human MLS/RCLS showed that high expression of the G1 cyclins, cyclin D and E, and their associated kinases CDK4 and 2, is a recurrent pattern in these tumors.

Furthermore, we have recently demonstrated, via luciferase assay experiments, the importance of NFkB for *Il.8* expression in *FUS-DDIT3* carrying cells, and we found a direct physical connection between the FUS-DDIT3 protein and nuclear IkBζ. The NFκB system controls genes involved in proliferation, migration and apoptosis. Modification of this system by FUS-DDIT3 would result in disturbance of vital functions in normal cells.

In summary, we have shown that the *FUS-DDIT3* fusion oncogene is capable of affecting multiple cellular processes and pathways. The potency of this fusion oncogene in liposarcoma development may be explained by this ability to affect several different cellular systems and thus induce multiple hits in the neoplastic pathway.

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