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Abstracts of Theses from the Nordic Countries

Short abstracts of theses on oncologic subjects are published under this heading. The abstract should contain background, problems, results and conclusions and be an independent informative unit that can be read without access to the thesis. It should not contain references to literature, figures or tables in the thesis. A suitable size is about 500 words. The abstract can be sent to Acta Oncologica together with information about department, faculty and university and date of dissertation.

Natural and induced immunity against the tumour-associated antigen, EP-CAM

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The tumour-associated antigen (TAA), Ep-CAM is overexpressed on various human carcinomas, including colorectal carcinoma (CRC).

TAAs or their immunodominant epitopes that are spontaneously recognised by the immune system might constitute a suitable target for immunotherapy. Fifteen per cent of sera of CRC patients with no previous immunotherapy elicited IgG antibodies against Ep-CAM. No Ep-CAM specific antibodies were detected in healthy controls or patients with Crohn's disease or colitis ulcerosa. Further analyses revealed that 50% of the Ep-CAM-reactive sera bound to peptide residues 29–46 of Ep-CAM. The results provide evidence for spontaneous immune recognition of Ep-CAM in CRC patients and identify an immunodominant B cell epitope of human Ep-CAM.

Anti-idiotypic antibodies (anti-Id) may serve as surrogate TAAs for vaccination. The optimal design of an anti-Id vaccine, however, remains unclear. Moreover, whether vaccination with anti-Id or the original antigen is superior is controversial.

SM262 is a human anti-Id raised against mAb 17-1A that recognises Ep-CAM. Vaccination of mice with anti-Id induced antibodies that shared idiotopes with mAb 17-1A and recognised Ep-CAM. Fusion of GM-CSF to anti-Id enhanced the magnitude of the antibody responses, while xenogeneic F_c domain had no significant modulatory effect. Recombinant anti-Id protein vaccine evoked a more potent humoral immunity as compared to DNA delivered by gene gun. Our study provides the first evidence that immune tolerance in mice expressing the transgene for human Ep-CAM can be circumvented by anti-Id vaccination. The results may have implications for future anti-Id vaccine design.

Vaccination of CRC patients with recombinant Ep-CAM protein, in combination with GM-CSF, induced Ep-CAM specific T and NK-like T cells producing cytotoxic cytokines. In addition, a long-lasting Th1 biased humoral and proliferate T cell response was elicited against Ep-CAM. The original antigen, Ep-CAM induced a more potent overall immune response as compared to anti-Id mimicking Ep-CAM. Analysis of TCR BV gene repertoire revealed that BV19+ CD8+ T cells might be involved in the vaccine induced anti-Ep-CAM immune response. The results collectively suggest that immunisation with Ep-CAM protein may serve as a novel approach to CRC immunotherapy. Furthermore, immunogenic MHC class I and II restricted Ep-CAM epitopes were identified that may provide new opportunities for developing effective multiepitope cancer vaccines targeting Ep-CAM.

Vaccination with a recombinant canarypox virus (ALVAC) encoding human Ep-CAM in combination with GM-CSF induced a potent Ep-CAM specific, type 1 cellular immune response in CRC patients. However, no anti-Ep-CAM antibody or proliferative T cell responses were elicited. Combining ALVAC-Ep-CAM and recombinant Ep-CAM in a prime-boost vaccination approach may represent an effective strategy to induce a coordinated antigen specific cellular and humoral immune response.

In conclusion, the results suggest that Ep-CAM is a promising target structure for immunotherapy. The present studies may form a basis for further enlarged clinical trials targeting Ep-CAM by active specific vaccination.

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Occupational exposure to electromagnetic fields and chronic diseases

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This thesis considers two exposures from the electromagnetic spectrum – extremely low-frequency magnetic fields (ELF MF) and ultraviolet (UV) radiation. ELF MF are the lowest and UV radiation ranges among the highest frequencies of non-ionizing radiation. The exposure prevalence of these fields is high in the general population. Most people are exposed daily to either or both types and potential health effects are of great concern. The aim of the thesis was to study occupational exposure to ELF MF and UV radiation in relation to cancer, neurodegenerative diseases and cardiovascular diseases. A cohort with an increased prevalence of individuals highly exposed to ELF MF was created in order to be able to study exposure-response relations. In all, four cohort studies and one case-control study were performed.

Exposure-response relationships were found for occupational ELF MF exposure and certain cancer diseases: malignant brain tumors among women, tumors of the pituitary gland among men. Welders were at an increased risk of tumors of the endocrine glands. The outcome might indicate an interaction with the hormonal system. A potential effect of occupational ELF MF exposure in cancer development cannot be rejected.

In the study of neurodegenerative diseases, the risk of both amyotrophic lateral sclerosis and Alzheimer's disease increased with increasing exposure to ELF MF. The results confirm previous findings of an association, and further epidemiological studies are needed, preferably based on morbidity data and taking into account potential confounding from e.g. electric shocks.

For acute myocardial infarction (AMI), a low level increase in relative risk was found as well as an exposure-response relation. Joint occurrence of ELF MF exposure and a genetic susceptibility for AMI showed a synergistic effect on AMI mortality. Effect modification from e.g. genetic predisposition to the disease deserves to be further explored in studies of ELF MF and AMI.

Swedish construction workers exposed to sunlight from outdoor work were at an increased risk of myeloid leukemia, lymphocytic leukemia or non-Hodgkin's lymphoma and stomach cancer. These findings are possibly due to an effect of UV radiation on the immune system. Sunlight exposure was also associated with an increased risk of malignant melanoma of the eye.

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Extensive research has been carried out in relation to ELF MF. There is yet no evidence of any biological mechanisms that could explain how ELF MF might contribute to cause chronic diseases. Therefore, additional efforts to explore potential pathways are warranted. Also, the question of effect modification from individual sensitivity such as genetic predisposition and interactions with other environmental factors deserves to be further explored.

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Pregnancy related risk factors for breast cancer

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Pregnancies offer a life-long reduction in breast cancer risk. It has repeatedly been shown that both number of pregnancies and age at first birth affect the future breast cancer risk. The mechanisms for this protection are still not fully investigated. Promising results from animal experiments indicate that the placental hormone hCG could be involved, but data from human populations are lacking. Likewise, it is still unclear to what extent pregnancy characteristics influence the protection. These are the questions addressed by this thesis.

The first study describes the risk of breast cancer after molar pregnancies. Molar pregnancies involve exposure to high levels of hCG, without other characteristics of a normal pregnancy. All women with a diagnosis of molar pregnancy in the Swedish Cancer Registry ($n = 3371$) between 1958 and 1993 were followed up for subsequent diagnoses of breast cancer. The observed number (59) was then compared to the expected number based on year of birth and follow-up period (46), yielding a SIR of 1.3 (95% confidence interval 1.0–1.7).

The second study uses another proxy variable for increased hCG exposure, namely hyperemesis. Breast cancer cases from the Swedish Cancer Registry were compared to matched controls regarding diagnoses of hyperemesis in the Swedish In-Patient Care Registry. There was practically no difference between cases and controls (adjusted OR = 1.05 for any versus no diagnosis of hyperemesis, 95% CI 0.86–1.27).

The third study focuses on duration of molar pregnancies and breast cancer risk. Women diagnosed with hydatidiform mole and a subsequent breast cancer diagnosis were identified from the Swedish Cancer Registry. They were compared to matched controls regarding the duration of molar pregnancy before evacuation, A slightly shorter period was noted for cases than for controls.

The fourth study investigates the possible relationship between abortions and breast cancer. Exposure information was abstracted from maternal care and birth records for 1759 cases and an equal number of matched controls. A history of at least one abortion was noted for 383 cases and 473 controls yielding an adjusted odds ratio of 0.84 (95% CI 0.72–0.99). Both induced and spontaneous abortions were associated with odds ratios below unity.

The last study investigates the impact of weight change during pregnancy, child weight and placental weight on maternal breast cancer risk, in the same material as study IV. A slightly increased risk was found with increasing child weight and placental weight, whereas no association was found with maternal weight change.

In conclusion, the presented studies do not support the theory of a protective effect of hCG on breast cancer risk. Further, abortions do not seem to increase breast cancer risk, at least not if followed by a childbirth. Finally, maternal risk of breast cancer seems to be modestly associated to offspring birth weight and placental weight, but not to maternal weight change during pregnancy.

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Multiparameter flow cytometry and minimal residual disease in patients with acute leukaemia

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The investigation of minimal residual disease (MRD) with multiparameter flow cytometry (FC) immunophenotyping has proven to be a powerful approach for disease monitoring in patients with acute leukemia (AL). This technique mainly relies on the identification of minor leukemic cell populations that can be discriminated from their normal counterparts on the basis of phenotypic aberrancies observed at diagnosis.

The immunophenotypic profiles exhibited by B- and T-cells in normal bone marrow (BM) and CD34+ cells in hematopoietic stem cells from blood collections (HSC-BC) have been established. Well-defined triple monoclonal antibody combinations showed patterns of antigen expression that corresponded to various maturation stages. Aberrant phenotypes were found in 98% of B-precursor ALL and in all studied T-ALL.

The identification of B- and T-cell subsets in BM allowed definition of 'empty spaces'. Areas of flow cytometric dot plots where no normal cell populations are located. Discrimination between normal and malignant cells can be done and used for MRD detection.

The immunophenotypic evaluation of MRD during therapy provides crucial information about the response to treatment and the risk of relapse. In 97% of 70 uniformly-treated children with ALL, the FC technique could be used for follow-up and detection of MRD. Patients with MRD levels greater or equal to 0.01% after Induction and the following time-point showed a significantly higher risk of relapse than patients with MRD values under 0.01% ($p < 0.05$)

Experience from the BIOMED-1, a European Concerted Action, contributed to an MRD evaluation in a standardised way, obtained with optimal quality and sensitivity.

Two main methods of MRD detection were compared: FC with 'live-gate' analysis and allele-specific oligonucleotide (ASO)-PCR, detecting patient-specific T-cells receptor γ/δ gene-rearrangements. The comparison showed significantly consistent results in 78% of the samples. BM samples taken during the first phase of treatment showed lower consistency when compared to samples taken during the later phase of treatment.

An investigation of MRD levels in HSC-BC and BM samples before collection and transplantation of hematopoietic stem cells was done. Low levels of MRD were found in HSC-BC from 24% of AL patients. MRD levels in HSC-BC correlated to the presence of MRD in the BM samples taken before collections. However, we could not find any correlation between the MRD levels in HSC-BC and outcome after hematopoietic stem cells from blood transplantation.

The investigation of levels and the dynamics of MRD by sensitive and quantitative FC technique can provide a basis for further clinical studies and decisions concerning patient therapy.

January 2004

New evidence on germ-cell testicular cancer aetiology

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Testicular cancer has been increasing in incidence for at least 50 years in many populations, but its etiology remains elusive. We investigated several prenatal and postnatal factors in association with germ-cell testicular cancer risk. Moreover, we evaluated recent trends in testicular cancer incidence in Northern European countries.

Using data from birth records and from the Swedish Cancer Register, we carried out a case-control study of 628 cases and 2,309 controls. We aimed at investigating the association between perinatal characteristics and testicular cancer, and assessing potential etiological heterogeneity between seminomas and nonseminomas, the two major histological groups of testicular cancer. Gestational duration was inversely associated with testicular cancer risk, whereas men with both high and low birth weight had an excess risk, indicating that intrauterine environment affects the risk of testicular cancer. Seminomas and nonseminomas seemed to have similar risk patterns.

Data from the first study were linked to the Swedish Military Service Conscription Register, which contains information on a medical examination that is mandatory in Sweden for the purpose of military service. The linkage permitted us to get information on body size at two different points in life on 371 cases and 1,238 controls. We found that height at eighteen years old is directly associated with testicular cancer risk. The association persisted after adjusting for perinatal characteristics, suggesting that both foetal life and later periods in life, such as childhood and adolescence, are important time windows for determining lifetime risk for testicular cancer.

A case-control study, including 3,051 cases and 9,007 controls born in Sweden after 1940, was carried out using data from the Multi-Generation Register and the Swedish Cancer Register. We obtained information on number and gender of first-degree relatives of all study subjects. We found that both low birth order and having few siblings is associated with an increased risk of testicular cancer. Since sibship size is correlated with birth order, we performed stratified analyses to disentangle between the effects of the two variables, and found that sibship size is a more important factor. We interpret that these findings are explained by an association between parental fertility and risk of testicular cancer in the offspring.

Data from the Multi-Generation Register and the Cancer Register were also used to investigate the fertility status before and after diagnosis of testicular cancer. Fecundity and the likelihood of fathering dizygotic twins, which is decreased among subfertiie subjects, were used as independent measures of fertility of 4,592 cases and 12,154 controls, born in Sweden in 1916 onwards. Prior to diagnosis cases had a decreased number of children, with a lower frequency of dizygotic twinning, indicating that testicular cancer patients have an increased frequency of fertility problems before diagnosis. After diagnosis cases fathered twins more often than controls, probably reflecting an increased use of assisted reproduction techniques.

Finally, the occurrence patterns of testicular cancer in eight Northern European countries were evaluated using data from national Cancer Registries. We found that the incidence of seminomas and nonseminomas is still increasing in all countries analyzed, with the possible exception of Denmark. Moreover, we found that, in Scandinavian countries, the increasing trend is a birth cohort phenomenon also in recent cohorts.

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Studies of VEGF-B and novel PDGFs in tumorigenesis and anagenesis

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The family of VEGF/PDGF growth factors and their receptors are important regulators of tumorigenesis and angiogenesis. VEGFs have been shown to play important roles in development of both blood and lymphatic vessels by effect on endothelia cells. PDGFs, on the other hand, are essential for normal function of perivascular cells, also for induction of tumor stroma reactions. This thesis work has focused on elucidating the expression pattern of two isoforms of VEGF-B and functional regulation of two novel PDGF family members, PDGF-C and PDGF-D in tumor growth and tumor angiogenesis.

Characterization of the expression pattern of two isoforms of VEGF-B, VEGF-B_{167} and VEGF-B_{186} in tissues and cell lines indicate that VEGF-B_{167} is the predominant isoform in most mouse tissues, accounting for about 80% of the total VEGF-B transcripts. VEGF-B_{186} is almost non-detectable within the same tissues. However, VEGF-B_{186} is up-regulated in mouse and human tumor cell lines, and primary tumors compared with their corresponding normal tissues. These data suggest a fine genetic control of the expression of two isoforms of VEGF-B, implying that VEGF-B_{186} might be involved in tumor development.

The novel PDGFs, PDGF-C and PDGF-D have a unique domain structure compared with the classical PDGFs, PDGF-A and PDGF-B. They contain a CUB domain besides the VEGF/PDGF homology domain. Importantly, the CUB domain has to be proteolytically removed before these factors could bind and activate their cognate receptors. We have focused on two issues, to characterize the role of the novel PDGFs during tumor development, and to identify the protease involved in activation of the novel PDGFs.

The tumorigenecity of the novel PDGFs were determined by their transformation efficacy in NIH/3T3 cells and in tumor cell lines. We identified that the novel PDGFs are potent transforming growth factors. The transformed cells displayed increased proliferation rate, anchorage-independent growth in soft agar, upregulation of VEGF, ability to induce tumors in nude mice. This data suggested that the novel PDGFs play an important role in cellular transformation and in tumor stromal reaction.

We identified the clot-busting protease, tissue-plasminogen activator (tPA), as a specific PDGF-C, but not PDGF-D, activating protease. The identification of tPA as an activator of PDGF signaling establishes a novel role for the protease, distinct from its well-known role in plasminogcn activation and fibrinolysis. Most importantly, it shed light on regulation control of PDGF-C in vivo.

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