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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.

Wnt-5a signalling in human mammary cells—Implications for the development of breast cancer

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The *Wnt-5a* gene encodes a secreted protein that regulates several normal processes in embryonic and adult tissues by as yet unknown mechanisms. Expression of Wnt-5a protein does not cause cell transformation, but it instead counteracts the effects induced by transforming Wnts. Inasmuch as Wnt-5a can activate the Wnt/β-catenin signalling pathway, we performed experiments to determine whether molecules participating in this pathway were modified in breast tumours. Thirteen percent of the tumours we studied showed accumulation of β-catenin protein but no mutations in the β-catenin gene or defects in APC protein. Moreover, the human dishevelled gene *(DVL-3)* was rarely activated in the examined tumours.

To elucidate the mechanisms of action of Wnt-5a, we studied the response of breast epithelial cells to overexpression or inhibition of this protein. Our results demonstrate that Wnt-5a is a co-factor that is needed to activate the collagen-binding discoidin domain receptor 1. In addition, the Wnt-5a protein exhibited activities involved in positive regulation of cell adhesion and negative regulation of cell migration. To determine whether the biological activities of Wnt-5a are linked to the development of breast cancer, we evaluated levels of this protein in invasive breast tumours. We found that expression of Wnt-5a had been lost in 44% of the studied carcinomas. This loss was significantly associated with higher histological grade and absence of estrogen and progesterone receptors, and proved to be an independent and powerful predictor of early relapse. Thus our data support the notion that Wnt-5a is a tumour suppressor, and that lack of this protein increases the risk of recurrent breast disease.

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Human papillomavirus and cervical carcinoma in situ—Implications for future screening

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The principal aim of this thesis was to study the temporal relationship between human papillomavirus type 16 (HPV 16) infection and cervical carcinoma in situ, particularly focusing on the effect of viral load. Other aims were to study the role of additional factors, especially smoking and oral contraceptive use, in cervical carcinogenesis.

We conducted a case-control study, nested within a population-based cohort of women participating in cytological screening in Uppsala county in Sweden during 1969 to 1995. All incident cases of cervical carcinoma in situ during this period were identified. For each case, one or two controls, matched by age and date of entry to the cohort, were selected randomly from the cohort.

A sensitive PCR assay was used to detect and quantitate HPV 16 load in repeatedly taken smears during up to 26 years before diagnosis in cases and their matched controls. Altogether 2553 archival smears from 495 cases and 2164 smears from 649 controls were tested. In addition, detailed information on sexual practice, smoking habits and oral contraceptive use were collected through telephone interviews with 422 cases and 422 controls.

We developed a single-tube nested PCR-based system for detection of 19 different genital HPV types in archival smears. Our detection system had a high sensitivity, comparable to the most commonly used PCR-based HPV typing systems.

We found an age-dependent increased risk of cervical carcinoma in situ in relation to current smoking, especially confined to women younger than 45 years. An increased risk was further demonstrated with increasing tobacco consumption (duration, intensity, pack-years). Current use of oral contraceptives was positively associated with the risk of cervical carcinoma in situ, with a monotonic increase in risk with increasing duration of use. The number of sexual partners was significantly, positively associated with risk among HPV 16/18-negative but not among HPV 16/18-positive women. We failed to demonstrate any increased risk in relation to age at first intercourse or parity.

The prevalence of HPV 16 positivity among cases was 56% at the time of diagnosis. Having an HPV 16-positive smear at entry to the cohort was associated with a moderately increased risk, whereas having persistent infection with HPV 16 in two subsequent smears was strongly associated with risk of cervical carcinoma in situ. We estimated that among HPV 16-positive women the median incubation period from first detected HPV infection to cervical carcinoma in situ was between 7 and 12 years.

Among cases, we found a consistently increased load of HPV 16, detected already 13 years before diagnosis, and often when the smear was still cytologically normal. Women with a high load of HPV 16, were at an over thirty-fold relative risk compared to HPV 16-negative women more than a decade prior to diagnosis. Women with the 20% highest viral load in their first smear, taken on average 7.8 years before diagnosis, had a sixty times higher risk compared to women negative for HPV in their first smear. We further estimated that approximately one-fourth of women infected with a high viral load before age 25 years, developed cervical carcinoma in situ within 15 years.

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Molecular studies of multiple endocrine neoplasia type 1 (MEN1)

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Multiple endocrine neoplasia type 1 (MEN1) is an autosomal dominant familial cancer syndrome characterized by tumors of the parathyroids, the endocrine pancreas and anterior pituitary.

The MEN1 locus has been previously localized to chromosome 11q13 and subsequently a MEN1 minimum region was defined by a combination of linkage and tumor deletion studies.

We performed cDNA selection on a bovine parathyroid library using a BAC containing *PYGM* from this region as a target, and isolated two novel genes termed *SCG1* and *SCG2*. We demonstrated that *SCG2* was ubiquitously expressed as a 2.9 kb transcript. Mutation analysis of this gene using SSCP on MEN1 individuals demonstrated the presence of inactivating frameshift, missense or nonsense mutations, which segregated with the disease. Accordingly, *SCG2*, subsequently named *MEN1*, was confirmed to be the gene responsible for MEN1. *MEN1* consists of 10 exons and encodes a 610 amino acid (aa) protein termed menin. Initial analysis of the predicted amino acid sequence revealed no homologies to any proteins or sequence motifs.

We then isolated and characterized the *Men1* gene in mouse. Murine *Men1* is smaller than its human counterpart, with a genomic size of 6.7 kb as compared to 9 kb. It contains 10 exons and is expressed co-dominantly as two transcripts, 2.8 and 3.2 kb. In situ hybridization analysis demonstrated that *Men1* was strongly expressed at gestational day 14, at a later gestational age strong expression becomes more restricted. In mouse, menin is a 611 aa protein, which shows 97% identity to human menin. Using a polyclonal antibody directed against human menin, we studied the expression of menin in both rat and mouse tissues in Western blot. A single band was seen in all tissue tested. Cell specific expression was seen, with menin localizing to the nuclei of nerve cells in the brain, while in testis the staining was perinuclear, using immunohistochemistry.

Using tBLASTX analysis, we identified and sequenced a zebrafish EST clone containing the *MEN1* gene, which when translated displays a predicted protein of 617 aa. When aligned with human and rodent menin, the zebrafish protein showed high conservation. It has 66.3% identity and 75.2% similarity to human menin. When we compared zebrafish menin to those amino acids which were previously shown to be affected by MEN1-associated missense mutations, we found 90% conservation (compared with 100% in rodent).

Menin expression was studied in human and primate cell lines, including lines derived from normal and MEN1-affected individuals. Menin was detected in nucleus and also in cytoplasm, albeit at lower levels. We found that the level of menin remained constant throughout the cell cycle, and displayed no size alteration. A strong signal at 68 kDa was observed in all lymphoblastoid cell lines, i.e. both normal and those carrying germline nonsense mutations. We did not detect a truncated protein nor was any obvious alteration in cellular localization or expression levels seen.

Finally, in an attempt to identify the 5′-end of the *MEN1* gene, we demonstrated strong heterogeneity of its transcripts, indicating that its expression is more complex than previously believed. Altogether six transcripts with alternative first exons were identified, three of which derived from the published intron 1, from a region which shows significant homology between human and mouse. They strongly overlap with the position of exon 1 in mouse *Men1* and may represent major *MEN1* transcripts in human.

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Ras-MAPK signaling in differentiating SH-SY5Y human neuroblastoma cells

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Neuroblastoma is a malignant childhood cancer, originating from sympathetic neuroblasts of the peripheral nervous system. Neuroblastoma is a heterogenous group of tumours, while some are highly malignant others can spontaneously mature into a more benign form or regress. Less than half of the patients survive and this statistics has improved only modestly over the past 20 years.

SH-SY5Y is a human neuroblastoma cell line established from a highly malignant tumour. The cells have retained a capacity to differentiate in vitro in response to low concentrations of the phorbolester 12-O-tetradecanoylphorbol-13-acetate (TPA) in the presence of serum or defined growth factors. Differentiated cells are characterised by neurite formation and upregulation of neuronal marker genes. SH-SY5Y are unresponsive to nerve growth factor (NGF), but when transfected to express the NGF-receptor TrkA, they differentiate in response to NGF. Protein kinase C (PKC) is pivotal for the differentiation response to take place.

We have investigated the role of signaling through the Ras-MAPK pathway in differentiating SH-SY5Y, with respect to neurite formation, expression of neuronal marker genes and growth control. Our results show that differentiation-promoting treatment induced a sustained activation and nuclear accumulation of the MAPK ERK in SH-SY5Y. The nuclear accumulation of ERK was PKC-dependent. However, nuclear accumulation of ERK was not sufficient for a differentiation response to take place in these cells, but ERK activity was needed for the characteristic upregulation of *NPY* and *GAP-43* induced by TPA. ERK activity did not induce neurite formation, neither was it necessary for TPA-induced neurite formation. Instead, stimulation of a pathway distinct from MEK/ERK, but downstream of Ras, was needed for morphological differentiation. We could also show that differentiated cells still entered S-phase and that there was no correlation between expression of the CKI $p21^{cip1}$ (an ERK target), BrdU-incorporation or neurite formation.

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Human ovarian surface epithelium and ovarian cancer—Aspects of growth regulation

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The ovary is covered with a single layer of epithelial cells, the ovarian surface epithelium (OSE). The OSE is degraded during every menstrual cycle to enable ovulation, and is subsequently reconstituted to cover the rupture site. This single cell layer is proposed as the origin for about 90% of all ovarian cancers. The active proliferative phases of the OSE after each ovulation is proposed to be the cause for its frequent transformation, since intensive cell growth enhances the risk for accumulation of mutations. The exposure of the OSE to high gonadotropin levels in postmenopausal women has also been suggested as an underlying factor, since the incidence of ovarian cancers increase during the postmenopausal period. Ovarian cancer has also been suggested to be a cytokine-propelled disease, since the ovarian cancer cells often both secrete cytokines, express cytokine receptors, and are growth stimulated by cytokines.

In this study, the levels of the chemotactic cytokine interleukin-8 (IL-8) was found to be about 13 times higher in cyst fluids from malignant ovarian tumors compared to cyst fluids from benign tumors or simple cysts. The IL-8 levels in plasma samples did not differ between patients with benign and malignant ovarian tumors, and thus this factor may be of limited clinical importance as an ovarian cancer marker. IL-8 was found to be expressed in both

epithelial tumor cells and stromal cells within the tumors, and the tissue levels of IL-8 increased with the malignancy of the tumors. The presence of both types of IL-8 receptors on ovarian cancer cells indicated a direct role for IL-8 on the tumor cells. In cultures of both normal human OSE cells and ovarian cancer cells, IL-8 was secreted into the media, and addition of a neutralizing antibody against IL-8, eliminating the autocrine stimulation, decreased the cell growth. These results demonstrate a possible tumor-promoting role for IL-8 in ovarian carcinomas.

The expression of IL-8 is partly regulated by the transcription factor C/EBPβ. This growth activating factor was also increased in the more malignant ovarian cancers, while the closely related antimitotic factor C/EBPα was constantly expressed in the different tumors, and its cytoplasmic location indicated a passive role for this factor.

OSE cells in culture was not found to be growth stimulated by either of the gonadotropins follicle-stimulating hormone (FSH) or luteinizing hormone (LH), a result that argues against gonadotropin-induced proliferation as a factor in ovarian cancer development. OSE cells was demonstrated to produce both estradiol and progesterone in vitro, showing that steroid production from ovarian, cancers is not a property acquired through transformation but a normal feature of OSE cells. Estradiol did not influence the growth rate of OSE cells in vitro, while the addition of a progesterone receptor blocker, Org 31710, increased the growth rate in OSE cells from postmenopausal cells. This indicates a growth inhibiting role of progesterone, which would be in line with epidemiological studies reporting a protective effect against ovarian cancer connected with the use of oral contraceptives and pregnancies, where progesterone levels are high.

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The role of physical activity in the prevention of breast and endometrial cancer

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The aim of this thesis was to explore through epidemiologic studies the role of physical activity in preventing breast and endometrial cancer in women.

First, we assessed risk for endometrial and breast cancer in relation to occupational physical activity in a large nationwide cohort generated through linkage between census data in 1960 and 1970 and the Cancer Register 1971–1989. We focused on women with the same level of estimated occupational physical activity in 1960 and 1970. During the nineteen years of follow-up, we observed a total of 1 949 cases of endometrial and 8 261 cases of breast cancer. We found a regularly increasing risk for endometrial cancer with decreasing level of occupational physical activity notably among women aged 50–69. Socioeconomic status did not appear to confound the risk estimates. The risk of breast cancer increased with decreasing level of occupational physical activity and with increasing socio-economic status. After adjustment for socioeconomic status, a 30% gradient in risk remained only among women 50–59 years old.

Second, we investigated the role of both leisure-time and occupational physical activity on endometrial and breast cancer risk in a nation-wide case-control study among Swedish women aged 50–74 years. Information on leisure-time physical activity during childhood, at ages 18–30 and in recent years as well as anthropometric, reproductive, and hormone-related factors was assessed through a detailed questionnaire. We estimated occupational physical activity through record linkage to the census data (1960–1990).

Among 709 women with endometrial cancer and 3 368 population controls, women with sustained low leisure-time physical activity were at a 50% higher risk of endometrial cancer than the most physically active women. Low occupational physical activity level during peri-menopausal years increased the endometrial cancer risk notably among women who were non-obese or smoked.

Among 3 347 women with breast cancer and 3 455 population controls, high leisure-time physical activity at ages 50–74 was associated with risk reduction. Women with sedentary occupations during their reproductive years had a 50% higher risk compared to those with the physically most demanding jobs. In comparison with active women, those with the combination of sedentary jobs and lack of leisure-time exercise had a 3-fold higher risk. The protective effect of occupational physical activity on breast cancer risk was limited to leaner women and more pronounced in those who never used hormone replacement therapy.

Third, we evaluated the effect of leisure-time physical activity during most period of reproductive years and occupational physical activity on breast cancer risk in a cohort of 11 408 female twins born 1886 through 1925. Information on physical activity, anthropometric measures, reproductive and social factors were assessed through a questionnaire. A total of 506 breast cancer cases occurred during follow-up from 1967 to 1997. Women with regular leisure-time physical activity had 20% lower risk for breast cancer compared with inactive women.

We found some evidence that the level of risk reduction was modified by birth cohort, age and body mass index in that the reduction in risk was more pronounced among older women with no overweight.

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