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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.

Epidemiological studies of host susceptibility in malignant lymphomas and colorectal cancer

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The aim of this thesis was to investigate the role of host susceptibility in malignant lymphomas and colorectal cancer. Specifically, the associations between infection and malignant lymphomas, skin cancer and survival from subsequent cancer, and the association between inflammatory bowel disease, familial disease, and colorectal cancer were assessed using Swedish population-based and nation-wide registers.

To investigate the role of chronic infections in the epidemic increase of non-Hodgkin's lymphomas, 5 199 individuals diagnosed or treated for tuberculosis 1939–1960 were followed up for cancer occurrence through 1996. Overall, there was a moderately increased risk of non-Hodgkin's lymphoma, with particularly elevated risks among patients with severe tuberculosis diagnosed before 1952.

The risk of Hodgkin's disease following infectious mononucleosis was assessed in one Swedish cohort of 21 510 individuals hospitalised with infectious mononucleosis 1964–1994, and in one Danish cohort of 17 052 individuals with a positive Paul-Bunnell test 1943–1978. The risk of Hodgkin's disease was more than doubled, increased with age at infectious mononucleosis, decreased with time since infectious mononucleosis, but remained elevated up to 20 years after the infectious mononucleosis. The highest risk was observed in the age-group 15–34 years.

The prognostic significance of a history of squamous cell skin cancer was assessed in five cohorts of patients with non-Hodgkin's lymphoma (n = 36 629), colon cancer (n = 77 039), breast cancer (n = 139 767), prostate cancer (n = 121 280), or lung cancer (n = 60 68l). In each cohort, the mortality among patients with a previous registration of squamous cell skin cancer was compared to that of same-site cancer patients without such history. In the cohorts, a history of squamous cell skin cancer was associated with a 20–30% increased risk of death.

To assess the significance of familial colorectal cancer and familial inflammatory bowel disease on the risk of colitis-associated colorectal cancer, 19459 patients with inflammatory bowel disease 1955–1995 were followed up for cancer occurrence 1958–1995. Familial colorectal cancer (assessed through record-linkage) was associated with a doubled risk of colitis-associated colorectal cancer, but familial inflammatory bowel disease had no significance.

To test the hypothesis of a common genetic determinant for inflammatory bowel disease and colorectal cancer, the risk of colorectal cancer among first-degree relatives of patients with inflammatory bowel disease was assessed. Among 114 102 first-degree relatives, no increased occurrence of colorectal cancer could be detected.

The results suggest that tuberculosis and infectious mononucleosis are risk factors for non-Hodgkin's lymphoma and Hodgkin's disease, respectively, that a history of squamous cell skin cancer is a marker of poor survival among patients with cancer, that inflammatory bowel disease and colorectal cancer do not share a common genetic cause, and that familial colorectal cancer (but not familial inflammatory bowel disease) is a risk factor for colitis-associated colorectal cancer.

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Cytogenetic heterogeneity and clonal evolution in the development of head and neck tumors

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Cytogenetic analyses of 235 short-term cultured head and neck squamous cell carcinomas (HNSCC) (130 oral SCC and 105 laryngeal SCC) revealed clonal chromosomal abnormalities in 121 tumors. The majority of these tumors displayed complex karyotypes with a nonrandom pattern of aberrations, leading to characteristic losses and gains of chromosomal segments. The most common genomic imbalances included gain of material from chromosomal arms 3q and 8q, chromosomes 7 and 20, and band 11q13, and loss of genetic material from chromosome arms 2q, 3p, 4p, 8p, 9p, 10p, 13p, 14p, 15p, and 22p, and chromosomes Y, 17, 18, and 21. Nearly half of the chromosomal breakpoints occurred in the centromeric regions, leading to the formation of isochromosomes and whole-arm translocations.

Cytogenetic heterogeneity was studied in 42 samples from 19 HNSCC. Intrasample or intersample heterogeneity, in the form of cytogenetically related or unrelated clones, was found in all but one tumor. By comparing the distribution of chromosomal imbalances in different subclones, it could be concluded that loss of 3p, 8p, 9p, and 18q and gain of genetic material from 3q and 8q are candidates for early genetic events.

A nasal papilloma and an acinic cell carcinoma of the salivary gland were cytogenetically investigated in detail after short-term culturing and in vitro passaging. Both cases showed extreme cytogenetic heterogeneity with 36 and 47 cytogenetically unrelated clones, respectively. During in vitro passage, all lines showed cytogenetic convergence i.e., the initial polyclonality was reduced to monoclonality with time. By molecular genetic techniques, it could be shown that cytogenetically unrelated clones had different cellular origins.

Samples of non-neoplastic upper aerodigestive tract (UAT) mucosa from 89 patients with or without HNSCC were analyzed cytogenetically. The cell cultures were divided into two series, depending on whether the cells were cultured in a medium favoring the outgrowth of stromal or epithelial cells. The results indicated that the frequencies of clonal and nonclonal chromosome aberrations in UAT mucosa are age-dependent, that cytogenetic support for the validity of the field cancerization hypothesis is restricted to increased levels of numerical chromosome changes

in epithelial cell cultures from cancer patients, and that clonal chromosome aberrations, including autosomal and sex chromosome aneuploidies as well as structural rearrangements, are not restricted to neoplastic mucosal cells.

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Estramustine-binding protein in brain tumours and normal brain

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In order to develop new efficient cancer treatments, it is important to understand the biology of tumours. A fundamental part of that process is related to protein composition in different tumours and in the surrounding normal tissue, especially proteins that interact with drugs. This thesis concerns studies of such a protein, estramustine-binding protein (EMBP), in brain tumours and in the surrounding normal brain. The feature that awoke the clinical interest in EMBP is its high-affinity binding of the cytotoxic drug estramustine (EaM), a combination of 17β-estradiol and nor-nitrogen mustard. Due to this binding, EaM is suggested to accumulate in EMBP-expressing tissues. EMBP was first discovered in rat ventral prostate, where it is a major protein. It was later found to be present, and bind EaM, both in human prostate and in prostatic cancer for which EaM-treatment is currently used. However, data about EMBP expression and EaM-binding in other tumour forms are accumulating.

In this thesis, EMBP is studied in glioma and meningioma. Immunohistochemistry and radioimmunoassay were used to study EMBP expression in tumour samples from patients with astrocytoma of different grades. High EMBP-levels were seen to correlate with poor outcome, although a low EMBP level did not always imply a favourable outcome. This finding suggests that EMBP may be a useful prognostic factor for patients with different grades of astrocytoma. Moreover, EMBP-expression was demonstrated in experimental rat glioma, with immunohistochemistry, Western blot, and reverse-transcriptase PCR, and in human meningioma with immunohistochemistry, Western blot and radioimmunoassay. Radioligand binding experiments were used to study EaM-binding characteristics, and Scatchard plot analysis of the results showed similar binding characteristics as prostatic EMBP. Accordingly, EMBP has been proposed to be involved in EaM-accumulation in glioma and meningioma. It was also shown that the levels of EMBP in glioma and meningioma were in the same range as in human prostate and prostatic carcinoma. Furthermore, the distribution of EMBP in normal rat brain was evaluated by immunohistochemistry. Prominent cytoplasmic staining was demonstrated in ependyma and leptomeninges, and nuclear staining was demonstrated in choroid plexus and the granular cell layer of the cerebellum. Cytoplasmic staining was also seen in large neurons throughout the brain. These findings may be important with regards to the effects of EaM-treatment. In the rat ventral prostate, and several other hormone sensitive glands, EMBP expression is androgen-regulated. There was, however, no major difference in EMBP expression between male and female rats in any of the tissues examined in this thesis.

In conclusion, the results presented in this thesis justify further studies regarding the role of EMBP in cells located in the central nervous system. Moreover, it is of interest to further evaluate the value of EaM in brain tumour therapy.

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Cytogenetic and molecular cytogenetic studies of uroepithelial carcinomas

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A series of uroepithelial carcinomas (bladder TCC and SCC, upper urinary tract tumors, post-radiation carcinomas, multifocal uroepithelial carcinomas, and bilharzia-associated bladder lesions) were examined by G-banding analysis, spectral karyotyping (SKY), fluorescence in situ hybridization (FISH), and comparative genomic hybridization (CGH).

The karyotypic profile of the tumors was characterized by nonrandom acquired chromosomal aberrations varying from one or few changes in low-grade and early stage tumors to massive abnormalities in muscle invasive ones. Nonrandom losses of chromosomal material dominated the karyotypic picture, with the single exception of post-radiation carcinomas. There was little intratumor cytogenetic heterogeneity and cytogenetically unrelated clones were never observed. A general correlation between the tumors' grade/stage and karyotypic complexity was evident. Invasive high-grade carcinomas were karyotypically more complex than low-grade superficial ones, indicating that progressive accumulation of acquired genetic alterations is the driving force behind multistep bladder transitional cell carcinogenesis.

Rearrangements of chromosome 9 resulting in loss of material from 9p, 9q, or of the entire chromosome were the most frequent cytogenetic alterations. These aberrations were seen as the sole change as well as in massively complex karyotypes, indicating that loss of TSG(s) from this chromosome is of major importance in uroepithelial carcinogenesis. The high frequency of chromosome 9 involvement in urinary tract transitional cell carcinogenesis may form the basis for a genetic marker to be used in the follow-up of patients with BC. Losses of material from chromosome 9 and from chromosome arms 1p, 11p and 8p, and gains of 1q and 8q seem to be early changes appearing in superficial tumors, whereas loss of material from 17p and the formation of an isochromosome for 5p were associated with more aggressive tumor phenotypes.

The analysis of upper urinary TCC revealed a karyotypic profile identical to that of bladder TCC, indicating that the same pathogenetic mechanisms are at work in both locales. Post-radiation uroepithelial carcinomas showed a distinct karyotypic and clonal pattern characterized by massive intratumor heterogeneity (cytogenetic polyclonality) and near-diploid clones with simple balanced and/or unbalanced translocations. G-banding analysis of secondary SCC of the bladder revealed indistinguishable karyotypes from the ones seen in advanced TCC of the same organ. The study of multifocal uroepithelial tumors revealed karyotypically related, often identical, cytogenetically complex clones in all tumors from each case, indicating that multifocal uroepithelial tumors have a monoclonal origin. Finally, although bilharzia-induced carcinomas showed cytogenetic profiles similar to the chemically induced ones in Swedish patients, our results indicate that bilharzia-associated tumors follow a more narrow pathogenetic pathway characterized by loss of material from 9p as the initiating event and gain of material from 19p as a later progressional event.

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Health of municipal sewage workers—Studies of cancer incidence, biomarkers of carcinogenicity and genotoxicity, and self reported symptoms

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The occupational exposures of sewage workers are complex and variable, and include a great variety of biological and chemical agents. Previous research has focused mostly on infections and various symptoms among sewage workers, e.g. abdominal and respiratory symptoms. At several sewage plants in Sweden, concern arose about occupational cancer, specifically cancer of the stomach, the kidney, and the lung. The aim of this study was to study the cancer incidence among municipal sewage workers, some exposures that might be connected with cancer risk, and self reported abdominal and respiratory symptoms.

In a cohort of municipal sewage workers there was no increase in the overall incidence of cancer when compared with the general population. However, there was a slight increase in the incidence of prostate cancer, but not in the sites of original concern among the workers. Infection by the gastric carcinogen *Helicobacter pylori* (determined from the presence of IgG antibodies in serum against *H pylori*) was no more prevalent in sewage workers than in comparable referents. Neither were sewage workers more exposed to genotoxic agents than comparable referents, as measured by the alkaline single cell gel electrophoresis (SCG) assay performed on peripheral lymphocytes.

There was no increase in the three-month prevalence of abdominal symptoms when compared with other municipal workers. Specifically, there was no difference in prevalence of the common disorders dyspepsia and irritable bowel syndrome. Sewage workers reported adult bronchial asthma significantly more than the referents.

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Aspects in mammographic screening—Detection, prediction, recurrence and prognosis

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Screening mammograms comprising of 32 first round, 10 interval and 32 second round detected cancers and 46 normal were examined by an expert screener, a screening radiologist, a clinical radiologist and a computer-assisted diagnosis (CAD) system. The expert screener, screening radiologist, clinical radiologist and the CAD detected 44, 41, 34 and 37 cancers, respectively, while their respective specificities were 80%, 83%, 100% and 22%. Later, with CAD prompting, the screening and the clinical radiologist detected 1 and 3 additional cancers each with unchanged specificities.

Screening mammograms comprising 35 first round, 12 interval and 14 second round detected cancers and 89 normal findings were examined without and with previous mammograms by experienced screeners. Without previous mammograms, the screeners detected 40.3 cancers with a specificity of 87%. With previous mammograms, 37.7 cancers were detected with a 96% specificity. The decrease in sensitivity was not significant but the screeners showed significant increase in specificity.

Local recurrences in 303 nonpalpable breast cancers with preoperative localizations and breast conservation therapy were evaluated for needle-caused implant metastasis. A total of 214 percutaneous biopsies were performed. There were 33 local recurrences. Needle-caused seeding or implantation as based on the location of the recurrence in comparison to the needle path in the mammograms was suspected in 3/44 (7%) invasive cancers without radiotherapy.

The mammographic characteristics of 317 nonpalpable breast cancers were categorized. Logistic regression showed that the risk ratios for a spiculated mass without calcifications and calcifications alone were 12 and 19 for invasive cancer and ductal cancer in situ (DCIS) respectively. Invasive ductal grade 1, ductal grade 2, lobular and ductal grade 3, had a risk ratio (RR) of 28, 17, 11 and 4.6, respectively, for a spiculated mass without calcifications. DCIS nuclear grade 3 and invasive ductal grade 3 had an RR of 17 and 9.7, respectively, for sole casting calcifications.

The eight-year survival of 96 1–9 mm invasive breast cancers were investigated in relation to their mammographic appearance, node status and histologic grade. After a median follow-up of 7 years, 6/96 died from breast cancer: 3/14 had calcifications alone, 2/56 had spiculated masses, 1/12 had rounded mass, 5/78 were node-negative and 1/4 was node-positive. The survival rate was 93%: 77% for the calcifications alone, 95% for spiculated masses, 91% for rounded masses, 92% for node-negative and 75% for node-positive. Calcifications alone and node positivity, each, carried a significantly higher risk of death.

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Studies on mechanisms of busulphan cytotoxicity and pharmacokinetics with special reference to liposomal busulphan

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Busulphan is an alkylating agent currently used in conditioning regimen prior to stem cell transplantation (SCT). High dose therapy with busulphan has been shown to contribute to transplantation-related toxicities, such as veno-occlusive disease (VOD) and interstitial pneumonia. Previous pharmacodynamic studies have attempted to define a target systemic exposure to busulphan, and thresholds for different side effects. Pharmacokinetic studies have shown a wide inter-patient variation in the systemic exposure to busulphan after a fixed dose. The wide variation may be due to bioavailability, age, underlying disease, drug–drug interaction and circadian rhythm.

The aim of the first part of this thesis was to develop and evaluate an intravenous formulation of busulphan encapsulated in liposomes. Small, uncharged liposomes containing cholesterol suspended in 5% glucose were selected for the preparation of liposomal busulphan (LBu). The distribution of LBu was assessed with ^{14}C-labeled busulphan in a rat model, and compared with a solution of free drug. The distribution of LBu was higher to bone marrow and spleen, and lower to brain, lung and heart, compared with free drug, while the distribution to liver was similar. The

myelosuppressive effect of LBu was stable and reproducible in a mouse model. Pharmacokinetics of liposomal busulphan was studied in man after either low dose or high dose of LBu. A linear relation between the systemic exposure and the dose was found. The pharmacokinetic parameters were in agreement with those previously reported for orally administered drug. It was feasible to use liposomal busulphan for high dose therapy. The advantageous distribution and good myelosuppressive effect in animals, and the more predictable pharmacokinetics in man have motivated the launching of a phase I/II clinical trial.

The aim of the second part of the thesis was to investigate mechanisms of busulphan-induced cytotoxicity, and the role of glutathione (GSH) in this process. The myeloid P39 cell line was used in vitro as a model for induction of differentiation and/or apoptosis with the differentiating agent all-trans retinoic acid and the cytostatic agent etoposide. Both agents induced apoptosis that was mediated through caspase activation, but different pathways were involved. These pathways diverged in kinetics, preceding maturation, cleavage of Bcl-2 and actin, and rescue from apoptosis by granulocyte colony-stimulating factor (G-CSF). Busulphan- and etoposide-induced apoptosis shared common features, but differed in kinetics. The P39 cells were arrested in G2 phase of the cell cycle before apoptotic morphology developed. Proliferation and clonogenic capacity of the P39 cells showed an inverse linear relation to the exposure to busulphan expressed as AUC. Also in busulphan-treated human CD34+ hematopoietic progenitors, clonogenic capacity was inversely and linearly related to the exposure to busulphan expressed as AUC. Myeloid progenitors were more sensitive than erythroid progenitors (AUCs completely inhibiting colony formation were 69 ± 7.5 μg.hr/ml and 140 ± 36 μg.hr/ml for CFU-GM and erythroid colonies, respectively). Neither an increase (induced by N-acetylcysteine), nor a decrease (induced by buthionine sulfoximine) of the cellular content of GSH affected busulphan-induced cytotoxicity in human CD34+ hematopoietic progenitors in vitro, or in murine bone marrow cells in vivo.

In conclusion, liposomal busulphan is a suitable formulation for high dose treatment in conditioning regimen prior to stem cell transplantation. N-acetylcysteine does not decrease busulphan-induced cytotoxicity in hematopoietic progenitor cells, and may thus serve as a potential prophylactic agent against transplantation-related hepatotoxicity.

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Effects of abdominal irradiation on neuronal VIP and substance P in the intestinal wall

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Radiotherapy is widely used as treatment for abdominal and pelvic malignancies: It is however correlated to side effects, such as nausea, vomiting, diarrhoea, malnutrition and abdominal pain. Abdominal irradiation is known to alter intestinal motility, derange intestinal mucosal integrity and to cause inflammation. Neuropeptides is a class of substances likely to be of importance during such processes. Two of the most relevant in the intestine are substance P (SP) and vasoactive intestinal peptide (VIP). SP enhances intestinal motility, increases blood flow and epithelial secretion and promotes inflammation. VIP induces smooth muscle relaxation, increases blood flow, is an antiinflammatory agent, stimulates epithelial secretion and promotes tissue growth and repair.

The aim was to evaluate the effects of irradiation on SP- and VIP-innervation in the intestinal wall in a short- and long-term perspective. Effects on SP and VIP contents in rat duodenal tissue were also examined, as were the effects on SP and VIP receptors/binding sites respectively. Duodenum from irradiated rats (5–30 Gy) and colon from patients irradiated for abdominal or pelvic malignancies were examined. VIP plasma levels were also analysed. Immunohistochemistry, receptor autoradiography, image analysis and radioimmunoassay were applied.

Distinct morphologic affection was evident in rat duodenum 7 days after exposure to 25–30 Gy. Ulceration and denudation was variously seen. Abundant nerve fibres showing SP- and VIP-like immunoreactivity (LI) were seen in lamina propria, especially in the most damaged regions. There was a twofold increase in VIP and SP levels in duodenal tissue after 30 Gy as compared with controls. In all animal groups, NK1-receptor (R) LI was detected mainly in epithelial cells and in the region of the interstitial cells of Cajal in the circular smooth muscle. In all groups, [^{125}I]VIP-binding sites were present in the smooth muscle and, with the exception of denudated regions in irradiated animals, in the mucosa. In irradiated human colon, the mucosal architecture was similarly affected. There were significant increases in SP- and VIP-LI nerve fibre numbers in lamina propria and circular muscle layer 4–7 days after irradiation. Intensified VIP- and SP-LI were seen in submucous ganglion cells. NK1-R-LI was detected in samples from the patients. There was no alteration in VIP plasma levels. At examination 5 weeks to 35 years after radiotherapy, the densities of SP- and VIP-innervation in human colon were decreased.

In conclusion, abdominal irradiation leads to increased intestinal SP- and VIP-innervation in the short-term perspective. Receptors/binding sites for the peptides are present in this radiation-affected tissue. In contrast, the SP- and VIP- innervation in the long-term perspective is decreased. As both SP and VIP have importance for intestinal physiology, the observations are of relevance for the understanding of intestinal processes after abdominal irradiation.

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Dynamics and gene expression of growth factor receptors in human cultured skin cells—Effects of UV radiation and calcium on EGF- and PDGF-receptors

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Growth factors transmit biological signals for the stimulation of cell growth and their stimulation may be involved in tumourigenesis. It is therefore of great importance to understand growth factor receptor reactions in response to stimuli such as calcium depletion or ultraviolet radiation, which normal human cells are invariably exposed to during their growth cycle.

We have studied human skin cells i.e. fibroblasts, keratinocytes and melanocytes and their growth factor receptor expression on

the surface of cells, reactions in the plane of the cell membrane, intracellular trafficking, and gene expression after UVB radiation, exposure to their ligand, serum, and calcium depletion. We have used fluorescence recovery after photobleaching (FRAP) to assess receptor characteristics in cell membranes, confocal laser scanning microscopy to visualize receptor internalization, ratio imaging for calcium studies, Northern blot for detection of the gene for the epidermal growth factor receptor (EGF-R) and flow cytometry for cell surface receptor determination. Platelet-derived growth factor receptors (PDGF-R) and EGF-R were studied since they both affect cell proliferation and viability.

Our results showed that the addition of PDGF increased receptor mobility characteristics in normal fibroblasts, also 'starvation' of cells increased their receptor mobility. We could show that changes in both intra- and extracellular free [Ca^{2+}] influence the mobility characteristics of PDGF-β2 receptors. We have demonstrated that the three cell types display different basal EGF-R mobility characteristics. After UVB irradiation mobility characteristics increased in all cell types but with differences in diffusion coefficients or mobile fractions. Addition of antioxidant enzymes; catalase and superoxide dismutase, prior to UVB irradiated cells abolished the UV-induced receptor mobility changes. We have shown that a single physiologic dose of UVB radiation alters the intracellular EGF-R distribution and intracellular transport in melanocytes. It also significantly alters the melanocyte phenotype. We were able to detect a constitutive EGF-R gene expression and showed that UVB radiation induces a time dependent induction in EGF-R mRNA in melanocytes. Human melanocytes express EGF-R on their cell surface and UVR induces time dependent changes in the number of receptors but the number of receptors does not correlate with the level of UV-induced EGF-R gene expression.

It is concluded that UV radiation, growth factors and calcium, ubiquitous constituents of every day life, all having tremendous effects in vivo, also affect human cells in vitro in parameters studied that are of importance for proliferation, survival and tumourigenesis.

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Molecular genetic studies on genes involved in hereditary nonpolyposis colorectal cancer (HNPCC)

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Colorectal cancer (CRC) is a major public health problem in Western countries, and it is the third leading cause of death from cancer in both males and females. Inherited predisposition can be divided into two subgroups based on the absence or the presence of multiple benign colorectal polyps. Hereditary nonpolyposis colorectal cancer (HNPCC) is one of the most common inherited cancer predisposing syndromes. It has an autosomal dominant transmission pattern, and it approximately accounts for up to 5% of all CRC cases. Individuals with HNPCC are also at an increased risk of developing cancers of the small bowel, biliary tract, uroepithelium, kidney, and central nervous system (CNS). So far, several mismatch repair (MMR) genes are known to be involved in HNPCC, and germline mutations have been identified in five of these genes, *hMSH2*, *hMLH1*, *hPMS1*, *hPMS2*, and *hMSH6*.

To determine and outline a mutation spectrum of MMR susceptibility genes as well as genes involved in CRC tumorigenesis pathway in the Swedish population, we have conducted a serial mutation analysis in big cohort of HNPCC or HNPCC-like families employing a number of molecular biological techniques. We have shown that 53% of the families that fulfilled stringent Amsterdam criteria harbor germline mutations in either *hMLH1* or *hMSH2*, while a lower mutation detection frequency (18%) was found in non-Amsterdam families. Based on our studies of specific tumor phenotype and genotype correlation in a big series of samples, as well as several clinical parameters, we have defined sensitive and specific criteria to be used in preselection for mutation screening. Various mutation detection techniques, both RNA- and DNA-based, were used. Our studies demonstrated the importance of confirming RNA variants in genomic DNA.

Two MutL homologous of *hPMS1* and *HPMS2* have been suggested to explain a few HNPCC patients. However, we did not identify any germline mutations in 84 HNPCC or HNPCC-like families in which the presence of other known MMR genes had already been excluded. Since no mutation in either of these two genes has been found to segregate with disease in 199 families studied so far, we conclude that it is possible that a mutation in *hPMS1* and *hPMS2* alone does not confer an increased risk for CRC. In a study in kindreds segregating endometrial and colorectal or endometrial cancer alone, a number of genes including all known MMR genes, *PTEN*, as well as *β-catenin* gene were investigated. Our finding of MSI negative tumors and lack of clear-cut germline mutation in any of the genes suggest no significant role for these genes, and imply that other genes are more likely to predispose to both endometrial and colorectal cancer in these families.

Single nucleotide substitutional mutations were commonly seen in MMR genes, especially in *hMLH1*. We have identified two such missense mutations in *hMLH1* and one in *hMSH2*. To determine whether these variants contribute pathogenic effects to CRC, we have investigated genotype–phenotype correlation in the families, studied the frequency of variants in general population, specific tumor phenotype as well as loss of heterozygosity (LOH). Our results indicated that the variant in *hMSH2* was considered a common polymorphism. In contrast, two *hMLH1* mutations were associated with an increased risk of CRC. Lack of microsatellite instability (MSI) in these tumors suggested that they might be involved in an unknown tumorigenesis pathway, other than those generally hypothesized.

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