Acta Oncologica 41, *No.* 5, *pp.* 486–495

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.

Germline *CDKN2A/ARF* alterations in human melanoma

JAMILEH HASHEMI

Department of Oncology/Pathology, Karolinska Hospital and Institute, Stockholm, Sweden

Approximately 10% of cases of human cutaneous malignant melanoma (CMM) have been estimated to occur in individuals with a familial predisposition, frequently in association with dysplastic nevus syndrome (DNS). The genetics of familial melanoma is complex and heterogeneous. To date only two melanoma predisposing genes have been identified. The *CDKN2A/ARF* locus on human chromosome 9p21 encodes two distinct cell cycle regulatory proteins, p16 and p14ARF. Germline alterations in the *CDKN2A* gene have been detected in approximately 20% of CMM families. Germline mutations have also been reported in the *CDK4* gene on chromosome 12q15, in a few families.

The primary aim of this thesis was to investigate alterations in the *CDKN2A/ARF* locus among individuals belonging to Swedish families with melanoma heredity and to study their biological consequences.

The relationships between germlinc *CDKN2A* mutations, melanoma and DNS phenotype were studied in five Swedish melanoma families with the mutations: a proline to leucine substitution (P48L), and an arginine insertion (113insR). We found significant correlations between *CDKN2A* mutations and CMM/DNS. Our results are consistent with the hypothesis that germline *CDKN2A* mutations and DNS both contribute to melanoma predisposition and may lead to early onset melanoma when present in the same individual.

The 113insR germline mutation was detected in 17 Swedish melanoma families. Haplotype analysis, using microsatellite markers in the 9p21 region, showed that these families share a common allele at markers close to *CDKN2A*, suggesting that 113insR is a founder mutation. Statistical analysis of meiotic recombination events in the 9p21 region indicated that the mutation arose approximately 2000 years ago.

We screened 80 individuals with multiple primary melanomas (MPM) for germline *CDKN2A* mutations. Mutations were detected in 11% of these patients and the majority of them had a family history of melanoma. A novel 24 base pair deletion, which included codons 62-69 (Δ62-69) was detected in one individual belonging to a family with melanoma heredity. *CDKN2A* mutation screening of individuals with MPM may thus identify high-risk families.

We confirmed the role of the P48L and Δ62-69 *CDKN2A* germline mutations in the development of melanoma tumors by demonstrating that the mutant p16 proteins are functionally abnormal and do not bind to CDK4 or CDK6.

Due to overlapping open reading frames in exon 2 of *CDKN2A*, the p16$^{\Delta}$62-69 also causes an in frame deletion of residues 77-84 in p14ARF. Our studies in cultured tumor cells showed that partial functional loss in p14ARF$^{\Delta}$77-84 may complement the defective p16Δ62-69 mutant and may contribute to melanoma development in patients carrying the 24 bp deletion in *CDKN2A*.

The high rate of *CDKN2A* inactivation in human cancer encouraged us to address the mechanisms and frequency of *Cdkn2a/b* gene loss during immortalization of *Ras*-transformed rat embryo fibroblast clones. We found homozygous deletions of *Cdkn2a/b* in all established cell lines studied. The mechanisms for loss of heterozygosity (LOH) were mitotic non-disjunction, deletion/rearrangements and, rarely, mitotic recombination. The frequency of *Cdkn2a/b* gene deletions was estimated to be approximately 2×10^{-8}/cell/generation.

March 2002

Sexual dysfunction and other distressful symptoms in cervical cancer survivors

KARIN BERGMARK

Department of Clincial Cancer Epidemiology, Institution for Oncology-Pathology, Karolinska Institute, Box 4402, SE-102 68 Stockholm, and Department of Gynecological Oncology, Institution for Oncology-Pathology, Karolinska Institute, Radiumhemmet, Karolinska Hospital, SE-171 76 Stockholm, Sweden

The majority of cervical cancer survivors are young or middle-aged women who will live many years with their treatment-induced sequelae. The effects of preoperative brachytherapy are unclear and treatment traditions vary—in Sweden and internationally. The relative occurrence of long-term distressful symptoms related to different treatments and the extent to which the women want to trade off optimal survival chances are not known.

The effects of radical hysterectomy were studied in a comparison with population controls, and the effects of additional brachytherapy could be studied due to various treatment policies at different centres. We used an anonymous postal questionnaire, studying the nature, occurrence and intensity of the symptoms and, separately, the corresponding symptom-induced distress.

We obtained information from 256 of 332 (77%) cervical cancer survivors and 350 of 495 (72%) population controls. Radical hysterectomy alone caused insufficient lubrication (relative risk (RR) 2.8 as compared to controls), reduced genital swelling at arousal (RR 1.5), reduced vaginal length (RR 6.1) and vaginal elasticity (RR 7.1), dyspareunia (RR 4.4), straining to void (RR 21.8), lymphoedema (RR 8.1) and distress from vaginal changes (RR 3.0). The addition of preoperative brachytherapy yielded RR 3.1 for defecation urgency, RR 8.5 for frequent nocturia and RR 1.6 for moderate and severe anxiety, but no excess risk concerning vaginal changes. The addition of external radiotherapy yielded, e.g., RR 13.1 for frequent nocturia and RR 4.8 for frequent defecation. A history of sexual abuse and cervical cancer gave RR 30.0 for superficial dyspareunia as compared to population controls with no history of sexual abuse. The majority of women were not prepared to forgo brachytherapy (even at a possible risk of 1% decreased survival) to avoid its long-term side effects.

Sexual dysfunction is the most distressful symptom in cervical cancer survivors, thus emphasising efforts to avoid it and interventions to relieve it. The excess risk of distressful treatment-induced symptoms from preoperative brachytherapy is low, if any, and the

majority of women prioritise optimal survival over freedom from treatment-induced symptoms. To meet the needs of women with early cervical cancer, a valid (randomised) study of the effects of preoperative brachytherapy is warranted. The long-term situation for cervical cancer survivors can be improved by clinical application of the data from this and other studies, and a number of areas for future research that may better the situation even more have been specified.

March 2002

Esophageal cancer evaluation of some new strategies

DAG STOCKELD

Division of Surgery, Karolinska Institute, Danderyd Hospital and Department of Oncology, Karolinska Institute, Radiumhemmet, Karolinska Hospital, Stockholm, Sweden

The objective was to study and to evaluate new strategies regarding the diagnosis, treatment and nutrition of esophageal cancer patients.

A background retrospective analysis of the files of all 1284 esophageal cancer patients in Stockholm County 1978–1995 was performed and followed by a multicenter study of the development and evaluation of a cisplatinum based chemoradiation protocol for the treatment of non-metastatic squamous cell carcinoma of the esophagus. An immunohisto-chemistry analysis of prechemoradiation biopsies from patients with squamous cell carcinoma of the esophagus regarding the expression of factor VIII, CD-34, p53 and bcl-2 and DNA ploidy was described. The results were correlated to tumor response. A method to register local spread of tumor in the esophageal wall through serial endoscopic fine needle aspirations (FNA). FNA was also evaluated as a diagnostic tool and the cytologic morphology was correlated to survival. Finally the technical aspects and risks of using percutaneous endoscopic gastrostomy (PEG) in a consecutive series of patients with esophageal cancer were evaluated.

A significant improvement in survival was found during 1978–1995. No survival benefit was noted for patients operated in high volume clinics. An increase in the incidence of adenocarcinomas among men was documented. Chemoradiation toxicity was mainly attributable to hematological impairment and lead to two adjustments of the treatment protocol (addition of filgrastim and lowering of the 5-fluorouracil dose). These changes made it possible to give the planned treatment to a gradually higher proportion of the patients. No correlations were found between the expressions of p53, bcl-2 or DNA ploidy and tumor response to chemoradiation. In spite of significant correlations to the markets for angiogenesis this significance may have been questionable since factor VIII had a positive and CD-34 a negative correlation to tumor response. In the FNA study a high proportion of patients had malignant cells in the esophageal wall at a considerable distance from macroscopic tumor. When the quotas between numbers of benign and malignant cells in tumor aspirates were evaluated, patients with a high quota had a median survival of 9.7 months while patients with low quota had a median survival of 23.2 months ($p < 0.03$). PEGs were successfully inserted in 97% after dilatation in 46%. The procedure related mortality was 0.9%. The right gastroepiploic artery was injured by the PEG in one surgical patient. One possible implantation metastasis was found.

Survival has increased among esophageal cancer patients in Stockholm County. The number of yearly esophageal cancer operations performed in a clinic did not correlate to long term survival. The chemoradiation protocol resulted in a combination of a high proportion of complete responses and, after protocol adjustments, a good compliance. The relapse pattern following chemoradiation showed that further development of the systemic part, i.e. chemotherapy, of the protocol might be beneficial. It was not possible to predict tumor response to the chemoradiation protocol studied through the analysis of pretreatment expression of p53, bcl-2 or DNA ploidy in biopsy specimens. In spite of significant correlations between complete tumor responses and the expressions of the markers for angiogenesis this significance may have been questionable since factor VIII had a positive and CD-34 a negative correlation to tumor response. Serial FNA could demonstrate local microscopic tumor spread in the wall of the esophagus in vivo in esophageal cancer patients. There may have been a correlation between the quota of benign/malignant cells in tumor aspirates in FNA and survival giving patients with a low quota a more favorable prognosis. PEG is a safe and a well tolerated way of ensuring enteral nutrition in patients with esophageal cancer.

April 2002

Molecular genetic analysis of chromosomal aberrations in DMBA-induced rat fibrosarcomas

ÅSA SJÖLING

Department of Cell and Molecular Biology-Genetics, Lundberg Institute, Göteborg University, Box 462, SE-405 30 Göteborg, Sweden

Cancer is a complex disease caused by alterations in several genes. It arises due to the stepwise accumulation of genetic changes in the descendants of tumor progenitor cells. During progression, the tumor cells acquire capabilities of enhanced growth and proliferation and ultimately they obtain the ability to invade adjacent tissues and to metastasize in other parts of the body. Genetic predisposition and environmental factors, such as diet, smoking, and exposure to carcinogenic compounds may promote initiation of tumors. Thus, the genetic events that ultimately lead to the formation of a malignant tumor are multi-facetted and complex. Consequently, some aspects of tumorigenesis may best be studied in a model system. In this study, first generation (F1; filial generation 1) animals from a cross of the inbred rat strains BN and LE were subcutaneously injected with the polycyclic aromatic hydrocarbon DMBA (7,12-dimethylbenz[a]anthracenc). Using fluorescent PCR amplification of informative microsatellite markers, DNA from the resulting solid tumors and tumor cell cultures were screened for allelic imbalance. The microsatellite marker alleles were compared pair-wise between tumors and normal liver DNA from each animal. Regions of recurrent allelic imbalance were detected on several rat chromosomes. The findings were compared to karyotype data and CGH (comparative genome hybridization) data from the same tumors. Based on the detected genomic aberrations, rat chromosome 1 (RNO1) was selected for further studies. The chromosomal changes in RNO1 appeared mostly to correspond to deletions of genetic material, but in one of the tumor cell cultures a bimodal pattern of amplified regions was detected by CGH. A method for of quantitation of allelic imbalance was used to delineate the positions of the two amplicons in the rat linkage map. Based on a comparative mapping approach, ten candidate genes from the distal part of RNO1 were investigated. The *Jak2* oncogene was found to be both amplified and overexpressed. The two amplicons had probably originated through repeated cycles of break-fusion-bridges, a chromosomal rearrangement that requires an initial

double-strand chromosome break, possibly at a so-called fragile site. Since loss of chromosomal parts leading to inactivation of tumor suppressor genes have been reported in the vicinity of double-strand DNA breaks, a detailed investigation of the region close to the initial break was undertaken. The *Pten* tumor-suppressor gene was shown to map close to the breakpoint. Analysis of a polymorphic *Pten* marker revealed heterozygous loss of one *Pten* allele in more than 60% of the tumors. This was a clear indication that *Pten* was selectively deleted in the fibrosarcomas, but the coding sequence of the remaining allele appeared to be normal. One plausible explanation for the absence of mutations may be that the tumor suppressive effect of *Pten* was haploinsufficient in our material. Thus, our tentativc interpretation of the findings is that treatment with DMBA induces, chromosomal breaks in RNO1, resulting in impaired *Pten* function and contributing to fibrosarcoma development.

April 2002

Hodgkin lymphoma—Studies of advanced stages, relapses and the relation to non-Hodgkin lymphomas

ROSE-MARIE AMINI

Department of Oncology, University Hospital, SE-751 85 Uppsala, Sweden

The relationship between Hodgkin lymphoma (HL) and non-Hodgkin lymphoma (NHL) is not entirely elucidated and a clonal relation may be present more often than previously believed. Mechanisms of tumour progression and resistance to therapy are poorly understood.

Between 1974 and 1994 all individuals in Sweden with both HL and NHL were identified. Thirty-two cases were studied using clinical, histopathological and immunohistochemical methods. The second lymphoma often appeared in an aggressive clinical form and a significant correlation between the expression of p53 and LMP-1 in the first and second lymphoma was demonstrated.

The treatment outcome for 307 patients with advanced stages of HL, in an unselected population, was in accordance with the treatment results of large centres world-wide. Some patients were successfully selected for a shorter chemotherapy-regimen without inferior treatment results.

In 124 patients with relapse, the survival of those primarily treated with radiotherapy according to the National guidelines was in accordance with the survival of patients of initially advanced stages. A worse outcome was found for those who received both chemotherapy and radiotherapy initially, probably because of a higher frequency of bulky disease in this group.

Immunohistochemical analysis of the tumour suppressor protein p53 and retinoblastoma protein (Rb) of paired samples at diagnosis and at relapse in 81 patients did not reveal any specific staining pattern affecting survival.

A novel B-cell line (U-2932) was established from a patient with a diffuse large B-cell lymphoma previously treated for advanced stage and subsequent relapses of HL. An identical rearranged IgH gene was demonstrated in tumour cells from the patient and in U-2932. A p53 point mutation was detected and over-expression of the p53 protein was found. A complex karyotype with high-level amplifications of the chromosomal regions 18q21 and 3q27, i.e. the loci for *bcl-2* and *bcl-6* were demonstrated.

April 2002

Hormonal treatments and the breast—Effects on sex steroid receptor expression and proliferation

ERIKA ISAKSSON FRIMAN

Department of Oncology and Pathology, Radiumhemmet, Karolinska Institutet, Karolinska Hospital, Stockholm, Sweden

Breast cancer is the most common malignancy among women in the western world. Hormonal treatments for contraception and replacement after menopause have been associated with an increased risk of breast cancer. The basis of risk associated with hormonal therapies may lie in the regulation of cell proliferation.

Normal breast tissue samples from 27 women undergoing reduction mammoplasties were analysed for sex steroid receptor content and proliferation. Women using hormonal contraceptives displayed a higher proliferation rate ($p = 0.05$) expressed as Ki-67/MIB-1 positivity than non-users. Proliferation displayed a strong positive correlation with IGF-I mRNA expression in breast tissue ($r_s = 0.82$, $p = 0.04$). During treatment there was a down regulation of ER values.

FNA biopsies were used to assess epithelial proliferation in normal breast tissue from 106 healthy premenopausal women with and without oral contraceptives. OC users had a higher ($p = 0.03$) proliferation rate (mean 4.8% of KI-67/MIB-1 positive cells) than non-users (mean 2.2%). Increased proliferation was associated with reduced levels of circulating androgens and free testosterone ($r_s = -0.38$, $p < 0.05$). There was a positive correlation between serum levels of both native progesterone ($r_s = 0.43$, $p < 0.05$) and the progestogen levonorgestrel ($r_s = -0.43$, $p = 0.02$).

Surgically postmenopausal cynomolgus macaques were treated for 35 months with either CEE, MPA, CEE/MPA or tamoxifen. Proliferation in breast tissue was associated ($p = 0.016$) with increased expression of p53. Tamoxifen was found to exert estrogen-like action in breast tissue by down-regulation of estrogen receptor expression and increased PRB levels. In the endometrium the lack of p53 expression in response to tamoxifen could be associated with an increased risk of endometrial cancer. Estrogen alone and estrogen in combination with progestogen was found to have different effects on the expression of sex steroid receptor isoforms in breast tissue. During combined CEE/MPA treatment both the ERβ/ERα and the PRA/PRB ratio had a tendency to be lower as compared to treatment with CEE alone. Suppression of the inhibitory isoforms ERβ and PRA may relate to increased proliferation during combined estrogen/progestogen treatment.

Ongoing HRT at the time of breast cancer diagnosis was found to influence certain tumor characteristics. Among 322 postmenopausal women with breast cancer 128 were using HRT at diagnosis. In this group the mean tissue concentrations of ER were lower (1.17 fmol/μg DNA) than in non-users (1.70 μg/DNA), $p < 0.05$. Different HRT regimens may have different effects which should be considered in the judgement on hormonal dependency and the clinical decision about endocrine therapy in women with breast cancer.

April 2002

The prognostic significance of uPA, uPAR and the cytokine IL-1α in urinary bladder cancer

MARIA SEDDIGHZADEH

Department of Oncology-Pathology, Karolinska Hospital and Institute, Stockholm, Sweden

Tumor invasion and metastasis are the major causes of cancer-related death, Proteases are important for the invasive and metastatic propensities of tumors. We have studied the mechanism underlying endogenous expression of the serine protease urokinase plasminogen activator (uPA) by human breast cancer cells. The expression of uPA mRNA was found to be affected by manipulation of the activity of the MEK-ERK signaling pathway. A minor portion of total ERK was phosphorylated (and hence active) in the MDA-MB-231 breast cancer cell line. Decreased ERK activity was associated with decreased uPA expression, whereas stimulation of ERK activity did not lead to an increase in uPA expression. In contrast, increased ERK activity was found to lead to increased expression of the cyclin kinase inhibitor p21Cip1. These data suggest that ERK activity in these cells is tuned to a level that allows rapid cell proliferation and high levels of uPA expression.

Urinary bladder carcinoma is heterogeneous in nature and can follow distinct clinical courses. More than fifty percent of all patients with muscle invasive bladder tumors that develop metastasis die from their disease. It is important to identify factors that can predict the prognosis of patients with muscle invasive tumors, both to be able to offer a more individualized treatment strategy and also to be able to develop therapeutic methods that can target these factors. In a prospective study we found that the expression of uPA and its receptor (uPAR) predict the outcome of patients with bladder cancer and that elevated levels of uPAR were associated with an increased risk for development of metastasis. Moreover, we observed that high uPA expression was associated with an increased risk of death from cancer in the group of patients with muscle invasive tumors. The risk associated with elevated uPA was lower in cystectomized patients, suggesting that treatment improved the prognosis. Elevated uPAR expression did not show this pattern.

Interleukin-1α (IL-1α) is a multifunctional cytokine with many potential roles in cancer. One such role is that of inducing expression of uPA in the tumor stroma. IL-1α has been shown to be expressed by bladder cancer cell lines and has also been shown to inhibit cell proliferation and angiogenesis in vitro. We observed that bladder tumor cells produce IL-1α but did not observe any correlation to uPA expression. Interestingly, low IL-1α expression was associated with a relative risk of 1.76 for death in cancer in the group of patients with muscle invasive tumors. The results indicate that uPA, uPAR and IL-1α can be used as markers for refined staging of tumors. Inhibition of the uPA system and/or treatment of patients with IL-1α may be considered as future therapy of urinary bladder cancer.

April 2002

Deregulation of the pRb pathway in human breast cancer

Martin Lodén

Department of Medical Biosciences, Pathology, Umeå University, SE-901 87 Umeå, Sweden

The retinoblastoma protein, pRb, pathway controls the G1/S transition by inhibiting E2F transcriptional factors, and S-phase is induced by repression of pRb function through sequential pRb phosphorylations by cyclin dependent kinases. In an attempt to identify subtypes of breast cancer and pinpoint patterns of cell cycle regulatory defects associated with clinical behaviour and altered proliferation, a multitude of cell cycle regulatory proteins were analysed in a material of 114 primary breast cancers.

These analyses demonstrated that the majority of human primary breast cancers have defects in G1/S transition proteins. Type of defects also differed in proliferative capacity and survival, and patterns of cell cycle defects might be used for prognostic purposes.

Cyclin E kinase activity correlated significantly with cyclin E content and inversely with p27 and p21 expression, suggesting that deregulation of cdk inhibitors and cyclin E acts through increased cyclin E kinase activity.

Cyclin E contents, but not pRb phosphorylation, correlated with proliferation. Consequently, the pRb pathway may not regulate proliferation in at least a subgroup of breast cancers and efforts were therefore made to search for alternative proliferation control mechanisms.

In ER positive tumours, cyclin D1 was associated with increased pRb phosphorylation and elevated proliferation. Tumours with high cyclin D1 were further associated with normal p27 expression and retention of wildtype p53, as well as older patient age. In ER negative tumours, proliferation was associated with increased cyclin E, but not with cyclin D1 or pRb phosphorylation, suggesting that pRb did not regulate proliferation in this group. ER negative tumours with high cyclin E were associated with downregulation of p27 and defect p53.

Upregulation of cyclin D1 or high cyclin E thereby seemed to represent two alternative mechanisms in the deregulation of the pRb pathway. However, tumours with high cyclin E had higher proliferative capacity and were associated with a more aggressive disease compared to tumours with high cyclin D1.

C-erbB2 inversely correlated with p27 contents. C-erbB2 was furthermore correlated with proliferation in tumours with linear pRb pathway i.e. tumours with preserved linearity between cyclin D1, pRb phosphorylation, cyclin E and proliferation, but not in ER negative tumours with elevated cyclin E. Therefore, c-erbB2 may have alternative roles in different tumours possibly based on type of pRb deregulative mechanism.

April 2002

Gonadal steroids in ovarian epithelial tumors

Peter Lindgren

Department of Clinical Sciences, Obstetrics and Gynecology, Umeå University, S-901 85 Umeå, Sweden

The primary role of the mammalian ovary is to release fertilizable oocytes. It also supports the reproductive process by the synthesis of steroid hormones which target both genital and non-genital tissues. The normally functioning ovary consists principally of three cell types: germ cells, stromal cells (endocrine and non-endocrine) and epithelial cells. It is estimated that 90% of ovarian cancers originate from the single layer of epithelial cells covering the normal ovary. Ovarian cancer is the fifth most common female cancer in Sweden and the leading cause of death from gynecologic malignancies. A relationship between ovarian epithelial cancer and gonadal steroids has been established through epidemiological and biomedical experimental research. The present investigation was undertaken to further characterize the possible role of gonadal steroids and their proposed local action on the origin, development and prognosis of ovarian epithelial cancer.

The mechanism of action of gonadal steroid hormones is mediated via ligand-specific receptors. Estrogen receptor alpha (ERα), estrogen receptor beta (ERβ) and progesterone receptor A/B isoforms (PR) were immunolocalized using mono- or poly-clonal antibodies in the nuclei of epithelial cells in normal ovaries and in

benign, borderline and malignant ovarian tumors of different histopathological types in both pre- and post-menopausal women. The expression of ERβ and PR was decreased in epithelial cells in tumors as compared with normal ovaries, based on mean indices of immunoreactivity scoring. All types of mucinous tumors (benign, borderline and malignant) retained ERβ expression, albeit at a lower level, a finding in sharp contrast to the loss of expression of ERα and PR in the same tumors. The development of epithelial ovarian cancer is dependent upon the balance between cell proliferation (tumor growth) and cell death. Immunolocalization of the proliferation marker, Ki67, and morphologic evaluation of programmed cell death, apoptosis, in women with poorly differentiated ovarian epithelial tumors demonstrated a relationship between high ERα expression, increased proliferation and decreased apoptosis. In primary ovarian epithelial tumor cell cultures, treatment with 17β-estradiol resulted in a reduced cell survival which was inversely proportional to hormone concentrations. Moreover, findings were suggestive of an autocrine and/or paracrine stimulation of 17β-estradiol release by epithelial tumor cells in culture. Ovarian tissue concentrations of 17β-estradiol and progesterone were hundred-fold higher than peripheral serum. In the present material of 81 consecutively sampled ovarian specimens, malignant ovarian tumors, when compared to benign ovarian tumors, contained decreased amounts of 17β-estradiol in both ovarian tissue and cyst fluid and decreased amounts of progesterone in ovarian tissue. Furthermore, in women treated for poorly differentiated ovarian epithelial cancer, those with elevated serum progesterone levels showed an improved 5-year survival, possibly further enhanced in combination with PR expression in the tumor.

Taken together, it is suggested that estrogens exert a facilitative role in ovarian epithelial carcinogenesis, an effect which may be counteracted by the local action of progesterone.

May 2002

The possible role of gut neuroendocrine peptides and amines in the pathogenesis and treatment of colorectal cancer

BASEL SITOHY

Department of Public Health and Clinical Medicine, Medicine, Umeå University, SE-901 85 Umeå, Sweden

Abnormalities in colonic neuroendocrine peptides/amines were observed in rats with chemically induced colon carcinoma. This supports the assumption that neuroendocrine peptides and amines of the gut seem to be involved in the carcinogenesis of colorectal carcinoma. These changes seem to occur prior to the development of colon cancer, since they appear at the dysplasia stage. The nature of the changes in the neuroendocrine peptides/amines in chemically induced carcinoma is, however, different from those observed in patients with colorectal cancer. This difference made this model unsuitable for studying the role of these substances in colorectal carcinoma. Treatment with a single therapy of octreotide or serotonin had no effect on rat colonic adenocarcinoma; galanin, however, reduced the relative volume density of blood vessels. Treatment with a double therapy of octreotide/galanin, octreotide/serotonin, or galanin/serotonin had certain effects on the rat colon cancer but not to the same extent as did the triple therapy. Triple therapy with octreotide, galanin, and serotonin reduced the volume and weight of the rat colonic cancer. This seems to have been brought about by increasing the tumor apoptosis and necrosis. The optimal dose for triple therapy with octreotide, galanin, and serotonin was between 10 and 20-μg/kg bodyweight. Doses under this limit had a marginal effect, while doses above this level did not increase the effect. No apparent side effects were found in mice during the period of treatment.

May 2002

A genetic study on familial breast cancer predisposing genes

LIPING LUO

Department of Molecular Medicine, Karolinska Institute, Stockholm, Sweden

Breast cancer is the most common malignant disease among women in the western world and 10% of all breast cancer is assumed to constitute hereditary cases.

Two major genes, *BRCA1* and *BRCA2*, can only explain a fraction of familial breast cancer. The *ATM* carriers have high risk of developing breast cancer. To evaluate the genetic roles of other susceptibility genes that may contribute to breast cancer, we used a PCR-SSCP method to screen for mutations in the *ATM* gene in sporadic and familial breast cancer cases. No somatic mutations were identified in the *ATM* gene in 38 sporadic breast cancer cases although LOH were detected in the region flanking *ATM* in 47% of the patients. Five constitutive rare polymorphisms were reported with a frequency between 0.005 and 0.23. A further study of 88 familial breast cancers revealed three germline mutations in the *ATM* gene. Two mutations had previously been found in A-T patients and one new mutation was a 1 bp insertion. Our studies rule out *ATM* as a major susceptibility gene for breast cancer.

To investigate the possibility of an epigenetic mechanism underlying the inactivation of the *ATM* gene in leukemia patients, where no mutations were found, DNA methylation in the promoter region of the ATM with 67 CpG islands was analyzed using bisulphite genomic sequencing. No hypermethylation was found in 9 T-PLL patients who did not show *ATM* or *Tp53* mutations. Six rare nucleotide substitutions in the *ATM* gene in T-ALL were detected, which were also identified in the germline DNA, indicating constitutive polymorphisms.

Another candidate susceptibility gene, *BACH1*, was studied for germline mutations by using PCR-SSCP in 29 breast cancer families exhibiting positive NPL with microsatellite markers flanking *BRCA1*, and an additional 95 breast cancer cases with a family history of breast cancer. No germline mutations were identified in any breast cancer patients. This result excludes *BACH1* as a frequently altered gene predisposing to familial breast cancer and does not support it as a breast cancer susceptibility gene.

Linkage analysis can pinpoint putative moderate penetrance genes and association studies are being used to search for low penetrance genes or other factors increasing the risk of developing breast cancer. A previous study used LOH to identify a candidate region on 17p in familial breast tumors and another study using comparative genome hybridization (CGH) pinpointed a region on 13q. To evaluate the potential contribution of these putative loci on chromosome 17p and 13q in 102 Stockholm breast cancer families without detected *BRCA1* and *BRCA2* mutations, we performed LOH study and targeted linkage analysis. Two loci with high frequency of LOH were found on 17p, one spanned the *Tp53* gene and the other was distal to *Tp53*. Linkage analysis either did not support any of the two loci on 17p as predisposing to familial

breast cancer. Nor was there any support for a putative breast cancer susceptibility gene on 13q.

May 2002

Evaluation of service screening with mammography in Sweden with special regard to its impact on breast cancer mortality

HÅKAN JONSSON

Department of Radiation Sciences, Oncology, Umeå University, SE-901 85 Umeå, Sweden

Female breast cancer is a common disease that is responsible for 10–20% of the total number of deaths in Sweden in the age group 35–59 years. Despite considerable effort to improve treatment, breast cancer mortality in many countries including Sweden has been constant over time. As there are no known risk factors suitable for effective primary prevention, efforts to reduce the number of breast cancer deaths have been directed towards mammography screening for early detection of the disease. National screening programmes have been launched in several European countries since the mid 1980s. The main objectives of this thesis were to evaluate the effects of the Swedish national service screening programme with mammography on the mortality from breast cancer and to develop models suitable for such evaluation.

In 1986 the National Swedish Board of Health and Welfare recommended service screening with mammography. The first counties launched screening programmes the same year and in 1997 the last county started screening. Service screening in Sweden is organised at the county level or sometimes at the level of smaller administrative units. Women aged 50–69 years were invited, although in many counties those aged 40–49 and 70–74 years were also invited. This led to differences among the counties in time for start and age limits, which made some geographical comparisons possible.

Data from the cancer register together with information on date and cause of death from the cause of death register were used. Information about screening characteristics within the counties was obtained by questionnaires sent to the screening centres.

Breast cancer mortality was only considered for cancer cases diagnosed after start of screening and for a certain age interval. Data from an earlier time period were used to adjust for possible baseline differences in breast cancer mortality. Two outcome measures were used, underlying cause of death as reported in the cause of death register and excess mortality. As a complement to crude relative risks based on the cumulative number of breast cancer deaths Poisson regression was also used.

Due to lack of individual data on invitation to screening a number of breast cancer cases were included that were diagnosed after the start of screening but before the individual invitation. This bias might have caused a dilution of the observed mortality reduction. Another bias was due to lead time because of the age limits. Adjustments were made for both these biases.

In a pilot study in northern Sweden two counties that started service screening in 1990 were compared with two counties without screening. A significant ($P < 0.05$) breast cancer mortality reduction of 0.28% was found for women 40–74 years.

Three of the studies focused on different age groups using data from all of Sweden In the age group 50–69 years breast cancer mortality was 20% lower in the counties that started screening early compared to those that started late. In comparing a group invited to screening with a group not invited to screening, a 12% reduction in breast cancer mortality was found for women aged 40–49 years. In women 70–74 years, an estimated 24% reduction of the excess mortality was found. The total numbers of person-years were 5.6, 33 and 1.8 million for the age groups 40–49, 50–69 and 70–74 years, respectively. The average follow-up time was 8–10 years.

A pilot service screening project started in Gävleborg county between 1974 and 1979. A long-term evaluation of the first 10 years of screening was performed with a follow-up of 22 years. The relative risk was estimated at 0.79 when compared to the neighbouring counties.

In conclusion, a reduction of breast cancer mortality related to service screening with mammography in Sweden was observed in all invited age groups.

The ratio between prevalence rate and expected incidence rate was studied for three randomised trials and service screening in six Swedish counties. An increasing ratio was found according to age, which is of interest regarding the much-debated question of the efficacy of mammography screening in different age groups.

May 2002

Prognostic factors in early stage cervical carcinomas treated with Wertheim-Meigs surgery

MARIANNE GRAFLUND

Department of Gynecological Oncology, Örebro University Hospital and Department of Biomedicine and Surgery, Division of Oncology, Faculty of Health Sciences, Linköping University, Linköping, Sweden

Cervical cancer is the second most common malignancy and a leading cause of morbidity and mortality among women worldwide. In Sweden, cervical cancer constitutes 2.4% of all newly diagnosed cancers and is the fifteenth cause of death.

An improved estimation of the prognosis in early stages of cervical carcinomas is desirable. The most important of the established prognostic factors are tumor size, radical excision margins, and lymph node status. The objectives of this study were to assess the value of oncogene and tumor suppressor gene products, angiogenesis, proliferation markers and histopathological malignancy grading systems as predictors of pelvic lymph node metastases (LNM), tumor recurrences and death due to the disease in early stage (FIGO I-II)

In a complete geographic series of cervical carcinomas treated by Wertheim-Meigs surgery, a number of clinical, biological, and histopathological prognostic factors were evaluated and long-term survival data were presented. In all, 367 woman with FIGO stage I-II cervical tumors were included.

Significant prognostic factors for disease-free survival were lymph node status, radical surgical margins, and tumor size. In a multivariate Cox analysis, it was shown that lymph node status was the single most important prognostic factor ($P < 0.0000001$). Presence of LNM, tumor recurrence, and death from disease were significantly associated with the FIGO stage. There was also a significant ($P = 0.002$) association between the vascular space invasion of tumor cells and the presence of lymph node metastases.

The complete malignancy grading system (MGS), partial index (PI), and invasive front grading (IFG) scones were highly significantly ($P = 0.0001$, $P = 0.0001$, $P = 0.002$) associated with the presence of pelvic LNM and with the disease-free survival rate. No pelvic lymph node metastates were encountered in tumors with MGS scores below 16. The predictive value (the specificity) for no pelvic lymph node metastates was 97%. The complete IFG score

and the individual scores of the two variables, pattern of invasion and host response, were all significantly ($P = 0.002$, $P = 0.007$, $P = 0.0001$) associated with pelvic LNM. Host response was the single most important factor in the IFG system, and it was superior to the complete score in predicting LNM.

The activity of the proliferation marker MIB-1 was lower in pelvic lymph node metastases than in the primary tumors. The expression of MIB-1 in lymph nodes was a prognostic factor for disease-free survival in both univariate and multivariate analyses.

In our series, it was concluded that microvessel density (CD31) and expressions of p53, bcl-2, p21 (WAF1), DNA ploidy, and S-phase fraction (FCM) did not add any further predictive or prognostic information.

In conclusion, this study has confirmed that histopathological malignancy grading (MGS), the partial index (PI), and invasive front grading (IFG) in the original or modified versions can predict low and high-risk groups of tumors and therefore be of value in planning the treatment of early stage squamous cell carcinomas of the uterine cervix. The expression of the proliferative marker MIB-1 in primary tumors and in LNM seems to be a factor that should be studied further in an attempt to identify different prognostic groups of tumors requiring more individualized postoperative treatment planning.

May 2002

Malignant mesothelioma—An experimental study with emphasis on proteoglycans in mesothelial cell growth and differentiation

KATALIN DOBRA

Department of Immunology, Microbiology, Pathology and Infectious Diseases, Division of Pathology, Karolinska Institute, Huddinge University Hospital, Stockholm, Sweden

Malignant mesothelioma is a highly aggressive tumor with median survival ranging from 4 to 12 months and, despite intense therapeutical efforts, it is invariably fatal. Mesothelioma cells are unique in the sense that they possess a biphasic growth potential and can be stimulated by serum growth factors to differentiate into stable epithelial or fibrous phenotypes. The prognosis of this tumor varies greatly depending on the differences in growth pattern, the most important predictor of poor prognosis being a fibrous phenotype.

To study the molecular basis of mesothelial differentiation, we used benign and malignant mesothelial cells in various stages of phenotypic differentiation. In order to evaluate the impact of proteoglycans (PG) on this process, a series of PGs were analyzed by semiquantitative reversed transcriptase polymerase chain reaction. The cells with epithelial phenotype showed increased expression of syndecan-2, syndecan-4 and hyaluronan synthase, and fibroblast-like cells expressed more matrix PGs: versican, decorin and biglycan. The PG profile may serve as a 'fingerprint', and reflect the maturation of mesothelial cells. The functional importance of syndecans in mesothelial differentiation was further shown by antisense targeting; down-regulation of each particular syndecan caused a loss of epithelial morphology, syndecans-1 and -4 being also essential for cell adhesion.

The differentiation of mesothelioma cells was influenced by treatment with various growth factors (TGF-β2, EGF, FGF-2, IGF-I and PDGF-BB). These factors affected the proliferation and morphology of mesothelioma cells to various extents, and the PG profile changed, in parallel, with an induced epithelial-mesenchymal transition. Exposure to EGF and IGF-I caused a fibroblast-like morphology simultaneously with a reduction in the syndecan expression levels. At the same time, the levels of shed syndecan-1 increased in the culture medium.

The involvement of other regulatory molecules in mesothelioma differentiation was assessed by subtractive hybridization, which has revealed a limited number of genes being differentially expressed between cells of epithelial or fibrous phenotypes. Most of these genes were recovered from the epithelial cells, which may indicate a more mature phenotype. The expression level of thioredoxin reductase, a small redox-active protein involved in drug resistance, was extremely high in both cell sub-lines, and may reflect the generic insensitivity of mesotheliomas to chemotherapy.

Although syndecans play a major role in regulating cell morphology, little is known about their subcellular distribution. Using confocal laser microscopy we found a substantial proportion of syndecans at intracellular locations, and syndecan-1 accumulated in the nucleus in a time-dependent manner. There was a close spatial relation of syndecans to tubulin in both interphase and mitotic cells. Vinblastine treatment interfered with the nuclear transport, and syndecan-1 and tubulin co-polymerize in paracrystalline occlusion bodies, in parallel with impaired nuclear transport. These findings suggest a tubulin-mediated transport mechanism. TGF-β2 reduced the proliferation rate of mesothelioma cells, concomitantly with a delay in nuclear transport of syndecan-1.

These data show that all syndecans are involved in maintaining the epithelial morphology, and that various amounts and translocation of syndecans may participate in molecular switches that regulate cell differentiation and proliferation. The above mechanisms may represent crucial steps, and possible future targets for therapy, that can be used to improve the management of patients with malignant mesothelioma.

May 2002

On prognostic and treatment predictive factors in early stage breast cancer

ANN-MARIE BILLGREN

Department of Oncology and Pathology, Radiumhemmet, Karolinska Institute, Stockholm, Sweden

Breast cancer is the most common malignant tumor in Swedish women and the incidence is increasing by 1–2% per year in the Stockholm area. A subgroup of patients is women diagnosed with primary inoperable disease. This group constitutes about 15% of all women affected by breast cancer. Primary systemic (neoadjuvant) therapy either with chemotherapy or endocrine therapy is frequently used in these patients.

Prognostication is especially important in identifying patients whose prognosis is so favorable that adjuvant systemic therapy is unnecessary. Prognostic factors can also be useful in identifying patients with poor prognosis that warrants an aggressive approach.

In the neoadjuvant setting the patients receive preoperative treatment and the behavior of the tumor during treatment may act as a biological model. Beside the earlier described prognostic factors additional factors such as clinical tumor response and early or late changes in biological markers during therapy may be of help in deciding the most beneficial therapy for the individual patient. However, the determination of a patient's clinical response to neoadjuvant therapy is sometimes difficult. An inaccurate response evaluation may prevent an early identification of non responders.

The conclusions are

Proliferation fraction (PF) (assessed in preoperative FNA biopsies) has a significant prognostic value which is independent of lymph node status, PgR status and tumor size. To our knowledge this is the first study demonstrating that PF can contribute prognostic information when analyzed in preoperative smears.

PAI-1 (assessed in surgical specimen) is a significant independent prognosticator independent of lymph node status.

A decrease in PF > 25% (assessed in fine needle aspiration (FNA) biopsies) during preoperative chemotherapy have a predictive value and may be of value in selecting postoperative adjuvant systemic treatment. In elderly parents, a high initial PF (assessed in FNA biopsies) may predict a decreased probability of response. Moreover, a decrease in the percentage of ER positive cells > 50% after 3 months tamoxifen therapy significantly predicted a lower probability of a long-term clinical response. Cathespin D (assessed in surgical specimen) may predict the benefit of tamoxifen amongst ER-positive patients. There is a poor correlation between clinically and mammographically assessed tumor size. Menopausal status, BMI and use of HRT are factors that could influence the correlation between the two assessments. Clinical assessment may not be the optimal method for response evaluation of preoperative systemic therapy. Mammographic assessment contributes with changes in size as well as density and gives a reproducible information.

May 2002

The role of human pappilomavirus in adenocarcinoma of the uterine cervix

Sonia Andersson

Division of Obstetrics and Gynecology, Karolinska Institute Danderyd Hospital, SE-182 88 Stockholm, Sweden

Cervical carcinoma is the third most common malignant disease in women worldwide. The absolute frequency of adenocarcinoma of cervix uteri has increased during the last decade and accounts for about 15–20% of invasive cervical cancers.

Human papillomavirus (HPV) is considered the most important factor in the development of cervical squamous cell carcinomas. HPV DNA has been identified in about 95% of cervical carcinomas. Adenocarcinomas of the cervix are also linked to HPV but the correlation is probably less pronounced.

The aim of this study was to analyse cervical adenocarcinomas with regard to the presence of HPV, the frequency of a specific mutation of the E6 gene of HPV 16 and the presence of p53 polymorphism, all of which may influence the risk of acquiring this carcinoma. Moreover, we intended to describe the symptoms and methods of detection of this tumour.

For the identification and typing of HPV, 173 cervical adenocarcinomas were analysed with Polymerase Chain Reaction (PCR), Single-Strand Conformation Polymorphism (SSCP) and/or DNA sequencing.

In HPV 16 positive cervical adenocarcinomas (28 cases), the E6 gene was analysed using the PCR-SSCP method for identification of the specific mutation (L83V). The results were correlated to those of 103 HPV 16 positive precancerous cervical lesions and 31 HPV 16 positive invasive cervical squamous cell carcinomas. For the analysis of p53 polymorphism, a PCR and SSCP based technique was used on 111 specimens and 188 controls (cell samples from women with normal cytology).

HPV was present in 68% of 173 adenocarcinomas. There was a significant correlation between the presence of HPV and age, illustrated by the fact that adenocarcinomas in younger women were more often HPV-positive than those in older women ($p < 0.001$). The most common types of HPV were 18, 16 and 45.

The HPV16 E6 variant L83V was present in 54% of the invasive adenocarcinomas. However, the predominance of the HPV16 variant observed earlier in squamous cell carcinomas was not seen in adenocarcinomas.

Homozygosity for arginine in codon 72 of the p53 gene was present in 71% of the adenocarcinomas but only in 47% of the samples from healthy female controls ($p < 0.001$). These results support the hypothesis that women with homozygosity for arginine in the p53 gene codon 72 may have an increased risk of developing adenocarcinoma of the cervix.

Our data demonstrate that 70% of the cervical adenocarcinomas were detected due to manifested symptoms (mainly vaginal bleeding) and 92% of the women tested with a Pap smear had normal results within three years before the cancer was detected.

HPV was identified in 68% of the cervical adenocarcinomas, and was predominant in younger women. Hence, the prevalence of HPV in women with cervical adenocarcinomas is age-related.

The most frequently occurring type of HPV was 18. Women with homozygosity for arginine of the p53 gene may have an increased risk of developing cervical adenocarcinoma. Since there has been an increase in the incidence of adenocarcinoma of cervix uteri among younger women, and Pap smear screening is inadequate for its detection, there is a need for other screening techniques. Repetitive HPV testing and analysis of p53 may help identify women at risk.

May 2002

The biological role and clinical impact of *SYT-SSX* fusion gene and IGF-1R in synovial sarcoma

Yuntao Xie

Department of Oncoloy and Pathology, Division of Cellular and Molecular Tumour Pathology, Cancer Center Karolinska, Karolinska Institute, Stockholm, Sweden

Synovial sarcoma, which is a highly malignant soft tissue tumour occurring mainly in young and middle-aged adults, is characterized by the translocation t(X; 18)(p11.2; q11.2). This translocation results in a fusion between the *SYT* gene on chromosome 18 and *SSX1* or *SSX2* on the X chromosome with the formation of new chimeric genes, *SYT-SSX1*, *SYT-SSX2*. The unique chromosomal translocation t(X:18)(p11.2:q11.2) is presented in more than 90% of the synovial sarcoma cases, and is believed to be an early and key molecular event playing a crucial role in tumour development and progression. This thesis aims to explore the biological role and clinical impact of *SYT-SSX* fusion genes in synovial sarcoma.

A novel fusion gene, *SYT-SSX4*, was identified in a case of typical synovial sarcoma, and harbours the same breakpoint regions as in *SYT-SSX1* or *SYT-SSX2*.

The potential relevance between the clinical outcome and *SYT-SSX* fusion types was investigated in 33 patients with primary synovial sarcoma. The 5-year metastasis-free survival for patients with *SYT-SSX1* was 42% vs. 89% for patients with *SYT-SSX2* with a hazard ratio (HR) of 7.4 (95% CI 1.5–36, log-rank $P = 0.004$). There was a significant association between *SYT-SSX1* and high tumour proliferation rate ($P = 0.02$). Furthermore, we found a significant correlation between *SYT-SSX1* and high expression of cyclin A and D1 in a large number of localized synovial sarcoma tumours. Those data suggest that *SYT-SSX* may influence the cell

cycle machinery, and that the more aggressive phenotype of the *SYT-SSX1* fusion type is due to an accelerated tumour cell proliferation.

Overexpression of IGF-1R was found in about 50% synovial sarcoma tumours. These tumours exhibited a significantly increased incidence of lung metastasis ($P = 0.01$). However, there was no correlation between the IGF-1R level and *SYT-SSX* fusion types, suggesting that they are independent of each other.

Antisense oligonucleotides (AS) to *SYT-SSX* mRNA expression rapidly and drastically decreased cyclin D1 expression and subsequently inhibited cell growth. The decrease in cyclin D1 expression was found to be primarily due to an increased degradation of the cyclin D1 protein via ubiquitin-dependent pathway, but was independent of the glycogen synthetase kinase-3β pathway.

In conclusion, this study suggests that *SYT-SSX* and IGF-1R are crucial for tumour cell proliferation and progression of synovial sarcoma, and may provide important predictive and therapeutic targets for this disease. Especially, a therapeutic targeting of *SYT-SSX* would be an ideal strategy since it would specifically ablate the tumour growth while sparing the normal tissues.

June 2002

Ovarian tumours, cell isolation, cell survival and sex steroids

MAGDALENA TAUBE

Department of Clinical Sciences, Obstetrics and Gynecology, Umeå University, Umeå, Sweden

Ovarian cancer is the most deadly malignancy of the female reproductive tract. Ovarian epithelial tumours are considered to be endocrine related, but the factors that govern their genesis and progression remain unclear. Radical surgery and recent advances in chemotherapy have only moderately improved survival rates. Biological and endocrine approaches may help to satisfy the pressing need for alternative therapies.

The aims of the studies included in this thesis were to isolate and culture ovarian tumour cells with a high epithelial cell fraction (ECF) and to evaluate the fluorometric microculture cytotoxity assay (FMCA) as a tool in the development of alternative therapies. We also aimed to measure the effects of bispecific antibodies (BsAbs) directed against three ovarian tumour markers, separately and in combination, and to study the effects of sex steroids on tumour cell survival, on autonomous steroid secretion and on the expression of estrogen (ER) and progesterone receptors (PR)

Tumour cells from a total of 75 patients were isolated and cultured in the experiments described by the four papers. The cells were incubated in the presence of BsAbs and peripheral blood mononuclear cells (PBMCs), and at varying concentrations of sex steroids. The FMCA and the neutral red cytotoxity assay were used to measure tumour cell survival. The time-resolved fluoroimmunoassay technique – DELFIA was used to measure steroid concentrations and immunohistochemistry was used to detect tumour markers, ER and PR.

It was shown that it is possible to isolate tumour cells from benign as well as malignant tumours, and that the fraction of malignant and atypical cells from carcinoma and borderline tumours can be determined in the cell preparation. Reliable predictive tests such as the FMCA performed on primary cell cultures can make it possible to create individual patient profiles, thus optimising drug treatment.

Analysis of the BsAbs cultures showed that a combination of the three BsAbs used in our study was the most efficient in tumour cell elimination. In some cases however, the effect of one single BsAb was better than the combination treatment, and this suggests that an optimal therapeutic approach based on BsAbs in a clinical setting requires a panel of BsAbs directed against the commonly expressed ovarian tumour markers.

Tumour cells cultured in 17 β-estradiol and testosterone showed a reduced cell survival and this reduction was inversely proportional to hormone concentrations within the range studied. It was found that 17 β-estradiol was secreted from the primary cell cultures and, interestingly, the amount of 17 β-estradiol secreted increased with increasing levels of 17 β-estradiol in the environment. The majority of the isolated cells analysed for ER or PR expressed both of these receptors to some degree, i.e. the combination ER + /PR + was the most common. Both ER and PR expression decreased after 72 h culture, revealing an unexpectedly dynamic system. Lowering levels of 17 β-estradiol in cell cultures reduced cell survival, but it appears that this observation depends on factors other than ER. The survival rates of cells cultured in progesterone seemed to be inversely related to their PR expression. It is believed that 17 β-estradiol has an antiapoptotic effect on ovarian surface epithelial (OSE) cells. Reduction of 17 β-estradiol in the environment may inhibit this effect, resulting in reduced cell survival. The ovarian epithelial tumour cell's ability to secrete 17 β-estradiol suggests that epithelial ovarian tumours play an active role in altering their own hormonal environment, promoting tumour progression.

September 2002