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Centre of Excellence

Division of Experimental Oncology

Department of Cancer Epidemiology
Department of Experimental and Clinical Radiobiology
Department of Medical Physics
Department of Molecular Biology
Department of Tumor Biology
and
Laboratory of Molecular Diagnostics and Radioimmunology
within Department of Nuclear Medicine and Oncological Endocrynology

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Scientific Report 2002-2004

Department of Cancer Epidemiology

The Department covers the following activities:

Cancer epidemiology studies

The analytical and descriptive studies are focused on:

- The rate analysis for the most frequent cancer sites (lung, breast and genitourinary organs) observed in Silesia District, classified by gender, age and period of calendar time.
- The oncocartography studying variations in cancer occurrence between different areas of Silesia District (on municipality level) or over time.
- An identification of important risk factors for selected cancer sites including lung, cervix and corpus uteri, breast, skin and larynx cancers.

The Regional Silesia Cancer Registry (RSCR)

RSCR is one of the 16 population-based cancer registries in Poland, covering a residential population of 4,9 million people living in highly polluted industrial Silesia District. RSCR routinely monitors cancer occurrence and aims to improve the quality and availability of the data. Together with data on death cases, the information collected by the registry is used to produce statistics about cancer incidence and mortality. It is a unique data resource for current and future research in cancer epidemiology. RSCR is fully computerized and routinely contributes the data to the National Cancer Registry. The registry is active in the collaboration with the Polish Association of Cancer Registries. In future, the work of cancer registry will expand from monitoring of cancer occurrence to the analysis of different aspects of cancer prevention, treatment and care.

The Bio-bank (in organization since September 2004)

It will fully cooperate with RSCR but its major objectives are:

- To provide a centralized, efficient and cost effective resource for receiving, processing and storing human biological materials for *ex vivo* research in biomedical disciplines.
- The Bio-bank will serve scientists from our Institute and other polish and foreign scientific institutions performing retrospective cancer case-control studies as well as molecular epidemiology projects.
- Apart from storing samples, the Bio-bank will offer additional services including: separation of whole blood samples into peripheral leucocytes and their fractions (e.g. mononuclear cells and granulocytes), red blood cells, and plasma; preparation of microscope smears of leucocytes or epithelial cells, DNA and RNA isolation as well as lymphocyte transformation in order to produce cell lines.
- The Bio-bank should facilitate communications between scientists with a variety of biological collections, which should make possible creation of new projects.

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Selected Papers:

Kennedy D. O., Agrawal M., Shen J., Terry M.B., Hang F.F., Senie R.T., Motykiewicz G., Santella R.M. ***DNA Repair Capacity of Lymphoblastoid Cell Lines From Sisters Discordant for Breast Cancer.***

Background: Interindividual differences in DNA repair capacity may influence cancer risk. We tested whether the nucleotide excision repair pathway was deficient in breast cancer case patients by analyzing sister pairs. *Methods:* Cell lines derived from sisters discordant for breast cancer (137 families containing 158 case patients and 154 control sisters) were obtained from the Metropolitan New York Registry of Breast Cancer Families. Lymphoblastoid cells were treated with benzo[*a*]pyrene diolepoxide (BPDE) for 30 minutes and were either harvested immediately or were washed and cultured in complete medium for 4 hours to allow DNA repair. Immunofluorescence using a polyclonal anti-BPDE–DNA primary antibody was used to quantify BPDE–DNA adducts. Percent DNA repair capacity was calculated from the difference between staining immediately after treatment minus that after 4 hours of repair, divided by the initial damage and was categorized into quartiles based on control values. Odds ratios and 95% confidence intervals (CIs) were calculated using conditional logistic regression models adjusted for age at blood donation, body mass index, and smoking. Statistical tests were two-sided. *Results:* Mean percent DNA repair capacity was lower in breast cancer case patients than in control subjects (difference = 8.6, 95% CI = 4.3 to 13.8, $P = .001$). Using the quartile with the highest percent DNA repair capacity as the referent group, adjusted odds ratios of breast cancer increased from 1.23 (95% CI = 0.57 to 2.65) to 2.38 (95% CI = 1.17 to 4.86) to 2.99 (95% CI = 1.45 to 6.17) ($P_{trend} = .002$) as DNA repair capacity decreased. *Conclusions:* Deficient DNA repair capacity is associated with increased breast cancer risk. *Journal of National Cancer Institute,* (2004), *in press*.

Juszko-Piekut M., Kołosza Z., Moździerz A. ***The influence of selected environmental factors on lung cancer incidence in immigrant population of industrial areas.***

Lung cancer risk factor in the immigrant population of an industrial area in Poland was examined in a case-control study. Both, relative and summary risks were calculated with adjustment for the following factors: cigarette smoking, alcohol consumption, atmospheric pollution in the place of residence, occupation, education and age. The multivariate logistic analysis indicated that cigarette smoking was a dominant risk factor. In addition, the following factors were confirmed to be significant lung cancer risk factors in the immigrant population of Upper Silesia (Poland): residence in the area of the greatest ecotoxins' concentration; work as toolmakers or manual laborers and seemingly, as coal-miners; lack of university or secondary education, and vodka consumption. Polish Journal of Environmental Studies, **13**, (suppl. II), (2004),174-180.

Juszko-Piekut M., Kołosza Z., Moździerz A. ***Epidemiological Analysis of Cutaneous malignant Melanoma Incicence in the Population of Upper Silesia***

So far, no detailed analysis of cutaneous malignant melanoma incidence in the population of Upper Silesia has been presented in literature. Thus, we evaluated the malignant incidence rates in male and female residents of the former province of Katowice in the 80s and 90s. Specific, crude and standardized rates calculated to evaluate the incidence of cutaneous melanoma. It has been documented that the melanoma incidence rates tend to increase in the population. On average, the standardized incidence rates in the years 1985-1998 increased by 5% per year in the men and 4% in women. Over the studied period, the increase in the incidence rates can be recorded in every younger groups, in both populations. It is observed that in men the neoplastic lesions mainly occurred on trunk or head and neck, where as they were concentrated on lower limbs and trunk in women. Polish Journal of Environmental Studies Vol.13, (Suppl. II) (2004), 181-187.

Zemła B., Banasik T.R., Tomaka A., Kołosza Z., Włodarczyk-Marciniec B.:
The epidemiology of breast cancer in female population of Silesia.

The incidence of the breast cancer in the years 1994-2000 in Silesia District was analysed. Age –adjusted incidence rates varied from 26.5/100 thousands to 47.8/100 thousands. The greatest breast cancer incidence among women is concentrated in the central part of Silesia District (the mostly industrialized). Such geographical distribution is caused by certain etiological background, which is not entirely clear yet. [Book published by Center of Oncology, Regional Silesia Cancer Registry and A.I.S.P.O Italy, Gliwice (2003), ISBN 83-909-137-4-7, *in Polish*]

Skowronek J., Zemła. B.: ***Epidemiology of lung and larynx cancers in coal mines in Upper Silesia – preliminary results.***

The results of the preliminary analysis of the risk of lung and larynx cancers among coal miners in Upper Silesia are present. The risk increases substantially during the work under condition of short-lived radon progeny hazard, especially when the concentration of alpha potential energy of short-lived radon progeny is higher than 2.5 ($\mu J\ m^{-3}$) that corresponds to the possibility of receiving the effective dose higher than 6 $mSv\ y^{-1}$. Significant differences of the risk are noticed between sub-populations of autochthon-miners and immigrant-miners: it was found that the relative risk for immigrant-miners was up to 2 times higher than for autochthon-miners. [*Health Phys.* **85** (2003) 365-370]

Szybiński Z., Huszno B., Zemła. B., Bandurska-Stankiewicz E., Przybylik-Mazurek E., Nowak W., Cichoń S., Buziak-Bereza M., Trofimiuk M., Szybiński P.: ***Incidence of thyroid cancer in the selected areas of iodine deficiency in Poland.***

The aim of the study was to evaluate the incidence rate (IR), trend and histotype of the differentiated thyroid cancer in the selected areas with varying iodine deficiency. The study was carried out in three areas: Krakow, (Carpathian endemic goitre area with 1,99 million mixed rural and urban population), Gliwice (Upper Silesia – moderate iodine deficiency area mostly industrial with 4,89 million inhabitants) and Olsztyn (slight iodine deficiency area, mainly rural with 0,77 million inhabitants). Between 1990 and 2001, in the study area 2691 newly diagnosed cases of malignant neoplasms of the thyroid gland were registered. In over 80% of patients it was differentiated thyroid cancer: mainly in women over 40 years, with F/M ratio 5,8. The highest percentage of papillary cancer 72,9% was observed in Olsztyn and lowest – 50,0% - in Krakow and Nowy Sacz districts. In the period of time incidence rate of differentiated thyroid cancer in women increased in Krakow, Gliwice and Olsztyn from 1,51 to 9,34 in 1998 1,27 to 5,74 in 1999 and from 2,52 to 11,35 in 2001 respectively. In the youngest (0-20 years) age group no significant increase of IR was observed. Between 1998 and 2001 the dynamics of increase of the thyroid cancer incidence markedly diminished. In conclusion it was hypothesised that an increase in IR of differentiated thyroid cancer in the study area was caused mainly by the suspension of iodine prophylaxis in 1980 and was diminished by an introduction of an obligatory model of iodine prophylaxis in 1996/1997. It was modified in terms of histotype and dynamics of increase by exposure to ionizing radiation. A very specific group at risk on the population level were women aged 20-40 years in the productive age exposed to iodine deficiency after suspension of iodine prophylaxis in 1980 and to radiation after the Chernobyl accident in 1986. [*J. Endocrinol. Invest.* **26** (Suppl.2) (2003) 63-70]

Zemła. B.: ***The epidemiology of malignant neoplasm of genitourinary organs in female and male population of Silesia.***

The objective of the study is: a) to show and to evaluate the female and male genitourinary system malignant neoplasm incidence frequencies (cervix uteri, corpus uteri, ovary, prostate gland, testis, as well as kidney and urinary bladder) taking into consideration the detailed age structure and the place of inhabitancy (i.e. according to 36 administrative units) in the years 1994-1999 (6 years) within the newly created Silesian Province (within the borders of 1999), b) the analysis of incidence changes, especially in comparison with the period 1985-1993 with reference to some of the above mentioned locations of neoplasm and some regions (the former Katowice Province and particularly the most industrialised part, that is the so called Upper-Silesian conurbation), c) the attempt to reconstruct (retrospective analysis) the relationships which existed or could or could not have existed between neoplasm pathology (pathogenesis), and the exogenous (geogenous, that is geographical-biosocial, for example atmosphere contamination, conditions of work microenvironments, ways of nutrition) based on own examination model which includes incidence risk in autochthonic and migrating populations, at least with reference to some pertaining organ neoplasm locations (cervix uteri and corpus uteri). [Book published by Center of Oncology, Regional Silesia Cancer Registry and A.I.S.P.O Italy, Gliwice 2002, ISBN 83-909-137-3-9]

Zemła B., Banasik T.R., Tomaka A., Kołosza Z., Włodarczyk-Marciniec B.: ***The epidemiology of breast cancer in female population of Silesia.***

The incidence of the breast cancer in the years 1994-2000 in Silesia District was analysed. Age –adjusted incidence rates varied from 26.5/100 thousands to 47.8/100 thousands. The greatest breast cancer incidence among women is concentrated in the central part of Silesia District (the mostly industrialized). Such geographical distribution is caused by certain etiological background, which is not entirely clear yet. [Book published by Center of Oncology, Regional Silesia Cancer Registry and A.I.S.P.O Italy, Gliwice 2003, ISBN 83-909-137-4-7, *in Polish*]

Department of Experimental and Clinical Radiobiology

The research interest of the Department is focused on molecular mechanisms of cellular response to ionizing radiation and other genotoxic factors, and on individual radio-sensitivity in human population.

The projects are connected with the following topics:

- Molecular and genetic background of radio-resistance and radio-sensitivity in human population
- Biological modifiers of in radiobiology and radio-oncology
- Proteins recognizing damaged DNA and their role in DNA repair
- Molecular characterization of terminal stages of apoptosis
- Molecular and cellular predictive and prognostic factors in radio-oncology
- Mathematical modeling in carcinogenesis and anticancer therapy

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(the laboratory is associated with Department of Radiotherapy)

Selected Papers:

Widłak P. ***DNA microarrays, a novel approach in studies of chromatin structure.***
The DNA microarray technology delivers an experimental tool that allows surveying expression of genetic information on a genome-wide scale at the level of single genes - for the new field termed functional genomics. Gene expression profiling - the primary application of DNA microarrays technology - generates monumental amounts of information concerning the functioning of genes, cells and organisms. However, the expression of genetic information is regulated by a number of factors that cannot be directly targeted by standard gene expression profiling. The genetic material of eukaryotic cells is packed into chromatin which provides the compaction and organization of DNA for replication, repair and recombination processes, and is the major epigenetic factor determining the expression of genetic information. Genomic DNA can be methylated and this modification modulates interactions with proteins which change the functional status of genes. Both chromatin structure and transcriptional activity are affected by the processes of replication, recombination and repair. Modified DNA microarray technology could be applied to genome-wide study of epigenetic factors and processes that modulate the expression of genetic information. Attempts to use DNA microarrays in studies of chromatin packing state, chromatin/DNA-binding protein distribution and DNA methylation pattern on a genome-wide scale are briefly reviewed in this paper. [Acta Biochim Pol. 51 (2004):1-8].

Weil MR, Widłak P, Minna JD, Garner HR. ***Global survey of chromatin accessibility using DNA microarrays.***
An increasing number of studies indicate a central role for chromatin remodeling in the regulation of gene expression. Current methods for high-resolution studies of the relationship between chromatin accessibility and transcription are low throughput, making a genome-wide study impractical. To enable the simultaneous measurement of the global chromatin accessibility state at the resolution of single genes, we developed the Chromatin Array technique, in which chromatin is separated by its condensation state using either the solubility differences of mono- and oligonucleosomes in specific buffers or controlled DNase I digestion and selection of the large refractory (condensed) DNA fragments. By probing with a comparative genomic hybridization style microarray, we can determine the condensation state of thousands of individual loci and correlate this with transcriptional activity. Applying this technique to the breast tumor model cell line, MCF7, we found that when the condensation is homogeneous in the population of cells, expression is inversely proportional to the level of accessibility and the two methods of accessibility-based target selection correlate well. Using functional annotation and comparative genomic hybridization data, we have begun to decipher the possible biological implications of the relationship between chromatin accessibility and expression. [Genome Res. 14 (2004): 1374-1381].

Fedorov A, Lukyanov D, Rogolinski J, Widłak P, Podgornaya O, Rzeszowska-Wolny J. ***The nuclear protein p30 specifically interacts with a nuclear matrix attachment region from the rat genome.***
In our previous study, a 454 bp DNA fragment was isolated from rat genomic DNA as an element which interacts with nuclear matrix proteins, i.e. a Matrix Associated Region (MAR). Computer analyses revealed that the right half of this fragment, named RME (Rat MAR Element), possesses a high matrix association potential and is likely to be responsible for the matrix association of the whole sequence. RME was used as a probe in an electrophoretic mobility shift assay (EMSA), and with the use of Southwestern blotting, a rat liver nuclear protein which binds specifically to it was identified. Its molecular mass was estimated by SDS-PAGE as 30 kDa (p30). Polyclonal antibodies raised against protein-RME complexes caused a super-shift of specific complexes in EMSA, and bound to p30 in nuclear extracts of rat liver in Western blotting. The immunofluorescence labelling of a rat embryonic fibroblast cell monolayer with anti-p30 antibody revealed a mainly intranuclear pattern of staining. [Cell Mol Biol Lett. 9 (2004): 153-165].

Łanuszewska J, Widłak P. ***The truncation of Ku86 in human lymphocytes.***
The Ku heterodimer, which consists of Ku70 and Ku86 subunits, is a major sensor of DNA breaks. A truncated form of Ku86 lacking its C-terminus, termed Ku86 variant, has been detected in extracts from different human cells. Here we report that in human lymphocytes the Ku86 variant is not present in vivo but is generated in vitro upon cell lysis by a trypsin-like protease. The resulting Ku86 variant exists exclusively in complexes with Ku70, which possess strong affinity to DNA double strand termini. In different blood donors the levels of Ku86 variant correlated with the magnitude of radiation induced DNA breaks. [Cancer Lett. 205 (2004):197-205].

Przybyszewski WM, Wideł M, Szurko A, Lubecka B, Matulewicz L, Maniakowski Z, Polaniak R, Birkner E, Rzeszowska-Wolny J. ***Multiple bystander effect of irradiated megacolonies of melanoma cells on non-irradiated neighbours.***

The multicellular megacolonies of human melanoma Me45 line growing on one part of the bottom of culture flasks were irradiated with 5 Gy (60Co), whereas megacolonies growing on the second part of the bottom were shielded. The bystander effect of radiation-traversed cells on non-traversed cells was studied during postradiation co-cultivation. Activity of superoxide dismutase (Mn and CuZn subunits), glutathione peroxidase (GSH-Pox) and malondialdehyde (MDA) concentration as a biochemical markers of bystander effect were monitored for a period of 72 h. The DNA damage was measured by the comet assay. Micronucleus induction, mitotic index and cellular death as apoptosis or necrosis were simultaneously estimated, based on morphologic criteria. The bystander effect of irradiated cells on their neighbours was observed as a slight increase of MDA concentration, comparable decrease of GSH-Pox activity, and some fluctuation of mitochondrial and cytoplasmic isoenzymes of SOD. DNA strand breaks and rejoining measured by comet assay as mean tail length, demonstrated clearly the bystander effect for nontraversed radiation cells, additionally verified by tail moment. There was also a significant increase of micronucleation and apoptosis generated by radiation traversed cells in shielded neighbours. Furthermore, significantly higher increase of necrosis in shielded neighbour cells compared to radiation traversed cells was observed. Proliferative activity showed a suppression in both, radiation traversed and shielded neighbour cells in all measured time points. The behaviour of used parameters points to the radical nature of modificators secreted by radiation traversed cells inducing bystander toxic damage in shielded neighbour cells. [*Cancer Lett*. **214** (2004): 91-102].

Konopacka M, Rogoliński J. ***Thiamine prevents X-ray induction of genetic changes in human lymphocytes in vitro.***

The effects of thiamine (vitamin B1) on the level of spontaneous or radiation-induced genetic changes in human lymphocytes in vitro were studied. Cultured lymphocytes were exposed to increasing concentrations of thiamine (0-500 microg/ml) and irradiated with X-rays. The DNA damage was estimated as the frequency of micronuclei and apoptotic or necrotic morphological changes in fixed cells. The results show that thiamine alone did not induce genetic changes. A significant decrease in the fraction of apoptotic and necrotic cells was observed in lymphocytes irradiated in the presence of vitamin B1 at concentrations between 1-100 microg/ml compared to those irradiated in the absence of thiamine. Vitamin B1 at 1 and 10 microg/ml decreased also the extent of radiation-induced formation of micronuclei. Vitamin B1 had no effect on radiation-induced cytotoxicity as measured by nuclear division index. The results indicate that vitamin B1 protects human cells from radiation-induced genetic changes. [*Acta Biochim Pol.* **51** (2004): 839-843].

Konopacka M**.** ***Effect of smoking and aging on micronucleus frequencies in human exfoliated buccal cells.***

In the present work the frequency of micronuclei (MN) in exfoliated buccal cells in 120 healthy individuals with relation to sex, age and smoking was investigated. Neither age nor sex showed any effect on the level of micronuclei. Smoking has shown a significant effect upon basal DNA damage. In the present study the calculated background frequency of micronuclei (per mille) in oral epithelial cells of 50 smokers and 70 non-smokers were 1.50 (+/-0.47) and 0.55 (+/-0.32), respectively. [*Neoplasma* **50** (2003): 380-382].

Kumala S., Niemiec P., Wideł M., Hancock R., Rzeszowska–Wolny J.: ***Apoptosis and clonogenic survival in three tumour cell lines exposed to gamma rays or chemical genotoxic agents.***

We compared the extent to which apoptosis is induced and clonogenicity reduced in three tumour cell lines - the human melanoma Me45 and promyelocytic leukaemia HL-60, and the rat rhabdomyosarcoma R1 - after exposure to the anticancer drugs etoposide and cis-platinum or to gamma radiation; each induces different types of DNA damage. Cells which readily underwent apoptosis did not necessarily show a correlated loss of clonogenicity; for example, Me45 cells showed the highest sensitivity to all three agents in clonogenic assays but much lower levels of apoptotic cells than R1 or HL-60 cells. These results show that the efficiency of the eradication of clonogenic cells by genotoxic agents does not solely depend on the induction of apoptotic processes, and suggest that the induction of apoptosis and suppression of clonogenicity are independent processes. [*Cell. Mol. Biol. Lett.* **8** (2003) 515–525]

Tarnawski R., Wideł M., Składowski K.: ***Tumor cell repopulation during conventional and accelerated radiotherapy in the in vitro megacolony culture.***

PURPOSE: To analyze the repopulation rate of cancer cells in vitro during conventional and accelerated irradiation, using the megacolony culture. MATERIALS AND METHODS: Two cell lines-murine squamous cell carcinoma AT478 and human adenocarcinoma A549-were grown as epithelial megacolonies in vitro, and they were irradiated using Co-60 gamma source at the dose rate of 0.82 Gy/min. Single-dose irradiation, conventional fractionation, and continuous accelerated irradiation (CAIR) were applied to determine the dose-response relationship and to calculate the repopulation balancing dose. Radiosensitivity parameters and the rate of repopulation were calculated from the colony cure rates using direct maximum-likelihood regression and a linear-quadratic model. Cytogenetic radiation damage was measured as frequency of necrotic, apoptototic cells and cells with micronuclei. Mitotic index was used as a simple measure of cell proliferation kinetics. RESULTS: When treatment time was increased, a significant drop in tumor control probability was detected. The loss of radiation dose calculated from LQ model parameters was equal to 0.8 Gy/day for both human and mouse cell lines. There was no evidence of a lag period for accelerated proliferation or altered proliferation during weekends. There were no significant differences in morphologic presentation of cellular radiation damage. CONCLUSIONS: In present in vitro experiments, we did not find any significant differences in repopulation or radiosensitivity between accelerated CAIR and conventional fractionation. Different mechanisms may be important for tumor cells repopulation in vitro and in vivo. [*Int J. Radiat. Oncol. Biol. Phys.* J **55** (2003) 1074-1081]

Wideł M., Jędruś S., Łukaszczyk B., Raczek–Zwierzycka K., Świerniak A.: ***Radiation – induced micronucleus frequency in peripheral blood lymphocytes is correlated with normal tissue damage in patients with cervical carcinoma undergoing radiotherapy.***

In an effort to find a test to predict the response of normal tissue to radiotherapy, the lymphocyte micronucleus assay was used on blood samples from patients with cervical carcinoma. Peripheral blood samples from 55 patients with advanced-stage (II B-IV B) cervical carcinoma were obtained before radiotherapy. The patients were treated with external-beam radiotherapy followed by high-dose-rate brachytherapy. Acute and late normal tissue reactions were scored and correlated with the micronucleus frequency in lymphocytes after irradiation with 4 Gy in vitro. Great interindividual variability was observed in the radiation-induced lymphocyte micronucleus frequency, especially at 4 Gy. The mean number of micronuclei per 100 binucleated cells in cells irradiated with 4 Gy in vitro was significantly higher in samples from patients who suffered from acute and/or late normal tissue reactions than in those from patients with no reactions (51.0 17.7 and 29.6 10.1, respectively). A significant correlation was also found between the micronucleus frequency at 4 Gy and the severity of acute reactions and late reactions. However, the overlap between the micronucleus frequencies of patients with high-grade late normal tissue reactions and low-grade reactions is too great to recommend the micronucleus assay in its present form for routine clinical application. [*Radiat. Res.* **159** (2003) 713-721]

Konopacka M., Rzeszowska–Wolny J.: ***Protective effects of vitamin C on radiation – induced DNA damage in cultured human lymphocytes.***

Cultured human lymphocytes were exposed to increased does of γ–radiation (1-4 Gy) and subsequently incubated in the presence or absence of vitamin C (10 μg/ml) and DNA damage was measured using the cytochalasin –B micronucleus test and the comet assay. The results demonstrate the ability of vitamin C to decrease in the frequency of radiation – induced micronuclei and DNA breaks. Protection against micronucleus induction by vitamin C was observed in cells exposed to γ – radiation at the dosages betwen 1-4 Gy, but the protective effect was less efficient at higher doses of radiation. Vitamin C post – irradiation tratment had no effect on apoptosis and cytotoxity as measured by NDI. The radiosensitivity of lymphocytes as well as the inhibitory effect of vitamin C calculated as a reversion factor differs among donors and ranged from 34%-53%. Vitamin C appears to be a useful candidate for the development of post – irradiation radioprotector. [*Human Monitoring for Genetic Effects. NATO Science Series I: Life and Behavioural Sciences*, vol. 351 (2003) 273-281]

Horak S., Polańska J., Widłak P.: ***Bulky DNA adducts in human sperm: relationship with fertility, semen quality, smoking, and environmental factors.***

The integrity of DNA of spermatogenic cells can be affected by endogenous and exogenous genotoxic factors. Resulting DNA damage in spermatozoa may significantly contribute to impaired fertility. Here, the 32P-postlabeling method was used to analyze the levels of bulky DNA adducts in sperm cells in a group of 179 males, either healthy donors or patients with an impaired fertility. When all donors were analyzed, the levels of bulky DNA adducts was 1.2-fold higher in smokers than in non-smokers, but the difference was not statistically significant ($P = 0.054$). However, a statistically significant difference existed between current smokers and never

smokers among the healthy individuals (1.7-fold increase, $P = 0.008$). No correlation between alcohol or coffee consumption and sperm DNA adducts was found. The levels of DNA adducts in sperm seemed to be unaffected by environmental and occupational factors. On the other hand, groups of healthy persons and patients with male-factor infertility differed significantly with respect to the level of bulky DNA adducts ($P = 0.012$). A significant negative correlation between DNA adducts and sperm concentration or sperm motility existed among patients with an impaired fertility ($n = 93$; $P < 0.029$, rS = -0.225). These results suggest that DNA adducts in sperm cells can be applied as potential biomarkers in studies of human infertility. [*Mutation Res.* **537** (2003) 53-65]

Walichiewicz P., Przybyszewski W.M., Jochem J., Wideł M., Koterbicka A., Śnietura M.: ***Inhibitory effect of local ischemic preconditioning in total body irradiated rats.***

The aim of this study was to explore the relationship between local ischaemic preconditioning and the effectiveness of fractionated radiotherapy. The rat serum, bone marrow, and small intestine were examined for oxidative changes induced by total body irradiation with gamma rays with applied local ischaemic preconditioning immediately before irradiation. Serum concentrations of TBA-RS examined 12 hours after the last irradiation did not reveal any differences among the groups of animals analyzed. Twenty-four hours after the last dose of irradiation, the serum concentrations of TBA-RS varied in particular groups ($P<0.0001$). The concentration of triglycerides in the serum of local preconditioned ischaemia and irradiated animals showed a reversed shape similar to the TBA-RS fluctuation ($P<0.003$). The level of uric acid in the serum of animals treated only with radiation is slightly higher than the level of this acid in the serum of the local preconditioned ischaemia radiation group ($P<0.58$). The number of bone marrow polychromatic erythrocytes did not appear to differ substantially in both irradiated groups. At the first 12 hours after irradiation, the frequency of micronucleated polychromatic erythrocytes is significantly different in the bone marrow of both groups either in combination with ischaemic preconditioned radiation or with radiation alone ($P<0.0002$). In irradiated animals without ischaemic preconditioning, on the 3rd day after irradiation the number of crypts increased and in the next days decreased achieving the level of the control group on the 7th day. Irradiated rats with local ischaemic preconditioning did not reveal an increase in the number of crypts. The difference was statistically significant ($P<0.05$). These data indicate that the local ischaemic preconditioning modifies the radiation peroxidising effects through inhibition of free radical-dependent lipid peroxidation and, probably, other unrecognized mechanisms. [*Teratogen. Carcinogen. Mutagen.* **23** suppl.1 (2003), 195-205]

Widłak P., Łanuszewska J., Cary R.B, Garrard W.T.: ***Subunit structures and stoichiometries of human DFF proteins before and after induction of apoptosis.***

DNA fragmentation factor (DFF) is one of the major endonucleases responsible for internucleosomal DNA cleavage during apoptosis. Understanding the regulatory checkpoints involved in safeguarding non-apoptotic cells against accidental activation of this nuclease is as important as elucidating its activation mechanisms during apoptosis. Here we address these issues by determining DFF native subunit structures and stoichiometries in human cells before and after induction of apoptosis using the technique of native pore-exclusion limit electrophoresis in combination with Western analyses. For comparison, we employed similar techniques with recombinant proteins in conjunction with atomic force microscopy. Before induction of apoptosis, the expression of DFF subunits varied widely among the cell types studied, and the chaperone/inhibitor subunits DFF45 and DFF35 unexpectedly existed primarily as monomers in vast excess of the latent nuclease subunit, DFF40, which was stoichiometrically associated with DFF45 to form heterodimers. DFF35 was exclusively cytoplasmic as a monomer. Nuclease activation upon caspase-3 cleavage of DFF45/DFF35 was accompanied by DFF40 homo-oligomer formation, with a tetramer being the smallest unit. Interestingly, intact DFF45 can inhibit nuclease activity by associating with these homo-oligomers without mediating their disassembly. We conclude that DFF nuclease is regulated by multiple pre- and post-activation fail-safe steps. [*J. Biol. Chem.* **278** (2003) 26915-26922]

Widłak P., Fujarewicz K.: ***The analysis of chromatin condensation state and transcriptional activity using DNA microarrays.***

The DNA microarray-based technique has been developed to semi-quantitatively measure the *in vivo* global chromatin condensation state at the resolution of a gene. Chromatin was fractionated due to the differential solubility of histone H1-containing and histone H1-free nucleosomes. A set of genes non-randomly distributed between histone H1-free (uncondensed or open) and histone H1-containing (condensed or closed) chromatin fractions has been identified. The transcript levels have been measured for the same group of genes. The correlation between transcriptional activity and chromatin fraction distribution of particular genes has been established. [*J. Med. Inf. Technol.* **6** (2003) IP13-IP19]

Konopacka M., Palyvoda O., Rzeszowska-Wolny J.: ***Inhibitory effect of ascorbic acid post-treatment on radiation- induced chromosomal damage in human lymphocytes in vitro.***
In the present study, the effect of exposure to ascorbic acid (vitamin C) after gamma-ray-induced chromosomal damage in cultured human lymphocytes was examined to explore the mechanism by which this antioxidant vitamin protects irradiated cells Non-irradiated lymphocytes were exposed to increasing concentrations of ascorbic acid (1-100 micro g/ml) and DNA damage was estimated using chromosomal aberration analysis and the comet assay. The results showed that ascorbic acid did not influence the frequency of chromosomal aberrations in non-irradiated cells, except at the highest concentration (20 micro g/ml), which induced breakage-type chromosomal aberrations. Vitamin C at the concentration of 50 micro g/ml caused DNA damage detected by the comet assay. A significant (34%) decrease in the frequency of chromosomal aberrations was observed in lymphocytes exposed to gamma-radiation and then cultured in the presence of ascorbic acid (1 micro g/ml). The removal of DNA breaks in cells exposed to 2 Gy of gamma-radiation was accelerated in the presence of ascorbic acid as determined by the comet assay, suggesting that it may stimulate DNA repair processes. [*Teratogen. Carcinogen. Mutagen.* 22 (2002) 443-450]

Przybyszewski W.M., Wideł. M., Palyvoda O.: ***Lipid peroxidation, DNA damage, and celluar morphology of R1 Rhabdomyosarcoma cell line irradiated in vitro by gamma-rays with different dose-rate.***
The study examines the relationship between lipid peroxidation, DNA damage, and cell morphology after the exposure of R1 Rhabdomyosarcoma cells to two different dose-rates of gamma rays. Exponential cultures of R1 cells were irradiated with single dose of 5 Gy at high dose rate (0.833 Gy/min) and low dose rate (0.0707 Gy/min). The concentration of two aldehydes, malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), were determined. DNA damage induction and repair were measured by using the alkaline version of the comet assay. Cellular alteration was also estimated microscopically as was the frequency of cells with micronuclei and proportion of apoptosis and necrosis. These parameters were evaluated immediately (time 0) and after different times up to 48 h of incubation in 37 degrees C, after irradiation. Results indicate that a low dose rate in comparison to high dose rate caused a significantly higher increase of aldehydes concentration observed at 12 h, followed by obviously higher DNA damage at 48 h and altered cellular morphology. The inverse dose-rate effect estimated for the gamma rays Co-60 source was found to be related to the measured biochemical and morphological parameters. [*Teratogen. Carcinogen. Mutagen.* **22** (2002) 93-102]

Palyvoda O., Mukalov I., Polańska J., Wygoda A., Drobot L., Wideł M., Rzeszowska–Wolny J.: ***Radiation-induced DNA damage and its repair in lymphocytes of patients with head neck cancer and healthy donors.***
BACKGROUND: DNA repair capacity may be an important factor in determining both individual susceptibility to cancer and the response to cancer therapy. The aim of this work was to compare DNA damage and the repair process in cells originating from healthy donors and cancer patients. MATERIALS AND METHODS: Using the micronucleus and comet assays, we compared the induction of DNA damage and its repair in lymphocytes isolated from blood samples of 14 healthy donors and 24 patients with head and neck tumours. Gamma-rays at the dose of 2 or 4 Gy were used as the damaging factor. The micronucleus test was performed according to Fenech (1) and the comet assay according to Green et al. (2). RESULTS AND CONCLUSION: Lymphocytes of both healthy donors and tumour patients showed great diversification in reaction to the same dose of gamma irradiation as well as differences in the kinetics of DNA repair. The patient group contained significantly more individuals whose lymphocytes were characterized by higher background DNA damage and higher damage inducibility. Blood cells of donors showing high damage inducibility also showed increased levels of micronuclei induced by ionizing radiation. Micronuclei induction did not correlate with a high level of unrepaired DNA damage. [*Anticancer Res*. **22** (2002) 1721-1728]

Walichiewicz P., Przybyszewski W.M. , Jochem J., Wideł M., Koterbicka A.: ***Inhibitory effect of local ischemic preconditioning on gamma ray-induced lipid peroxidation in rats : a preliminary study.***
We examined the effect of local ischemic preconditioning on postradiation lipid peroxidation in the serum of total body irradiated rats. Markers of peroxidative damage provoked by radiation alone or radiation preceeded by ischemic preconditioning were thiobarbituric acid reactive substances, triglycerides and uric acid concentrations in serum. These data indicated that local ischemic preconditioning modifies the peroxidizing effects of radiation through inhibition of free radical-dependent lipid peroxidation. Other unrecognized mechanisms are probably also involved. Uric acid could act as an antioxidant against radiation alone and local preconditioned ischemia together with radiation. [*Int. J. Tissue React*. **24** (2002) 143-150].

Beck E., Polaniak R., Wideł M., Drzazga Z.: ***Influence of electromagnetic field on murine sqamous cell carcinoma cells in vitro.***
An influence of extremely low frequency electromagnetic field on the growth of murine squamous cell carcinoma cells of the line AT478 was studied. The preliminary results indicate that applied electromagnetic field can by cytotoxic and can induce genetic damage in the prolonged time. Observed effects depend on the time of exposure to the applied electromagnetic field. [*IFMBE Proceedings* **2 (2**002) 142-143]

Wideł M., Lubecka B., Tarnawski R.**,** Czuba. A: ***Fractionated radiotherapy of tumour megacolonies: Cytogenetic, flow cytometry and survival studies.***
Multicelluar megacolonies of human lung adenocarcinoma, A549 and murine squamos cell carcinoma, AT478 were treated with conventional fractionation (CAIR) up to graded does 20-80 Gy. Tumour control doses (TCD_{50}) estimated on the base of megacolony cure rate and clonal regrowth were about 12Gy higher for CF than for CAIR. Cytogenetic analysis of micronuclei, apoptosis and nacrosis frequency indicated that all these types of celluar damage were considerably higher after similar accumulated doses given in continuous than in conventional schedules. Results indicate that repair of damage and repeated repopulation during weekend-breaks lead to lower efficiency of conventional treatment in comparision with CAIR. [*IFMBE Proccedings 2* (2002) 190-191]

Widłak P., Palyvoda O., Kumala S., Garrard W.T.: ***Modeling apoptotic chromatin condensation in normal cell nuclei.***
Hallmarks of the terminal stages of apoptosis are genomic DNA fragmentation and chromatin condensation. Here, we have studied the mechanism of condensation both *in vitro* and *in vivo*. We found that DNA fragmentation *per se* of isolated nuclei from non-apoptotic cells induced chromatin condensation that closely resembles the morphology seen in apoptotic cells, independent of ATP utilization, at physiological ionic strengths. Interestingly, chromatin condensation was accompanied by release of nuclear actin, and both condensation and actin release could be blocked by reversibly pretreating nuclei with Ca, Cu, diamide, or low pH, procedures shown to stabilize internal nuclear components. Moreover, specific inhibition of nuclear F-actin depolymerization or promotion of its formation also reduced chromatin condensation. Chromatin condensation could also be inhibited by exposing nuclei to reagents that bind to the DNA minor groove, disrupting native nucleosomal DNA wrapping. In addition, in cultured cells undergoing apoptosis, drugs that inhibit depolymerization of actin or bind to the minor groove also reduced chromatin condensation, but not DNA fragmentation. Therefore, the ability of chromatin fragments with intact nucleosomes to form large clumps of condensed chromatin during apoptosis requires the apparent disassembly of internal nuclear structures that may normally constrain chromosome subdomains in nonapoptotic cells. [*J. Biol. Chem*. **277** (2002) 21683-21690]

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Laboratory of Biophysics

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The laboratory brings together physicists, biologists and chemists engaged in the studies of brain biochemistry using NMR spectroscopy. Research is focused on the studies of intact biological systems by MRS in vivo and the high resolution in vitro NMR model studies of animal brain extracts and cell lines.
The main interests ivolve brain tumor metabolic profiles, radiotherapy monitoring, brain metabolic disturbances in adult and juvenile MS and drug refractory epilepsy.

Laboratory of Dosimetry and Quality Control in Radiotherapy and Rentgenodiagnostics

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The focus of the research in this laboratory is the clinical implementation of improved treatment and verification methods, like portal imaging and in vivo dosimetry.

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Selected Papers:

Sokół M, Przybyszewski W, Matlas B. ***Investigation of metabolic changes in irradiated rat brain tissue by means of 1H NMR in vitro relaxation study***

The effect of irradiation on concentrations and relaxation behaviour of brain metabolites was studied by means of high-resolution ^{1}H NMR in vitro. The studies were performed on rat brains irradiated with the doses of 20 Gy applied in fractions of 2 Gy. Standard procedures were used to obtain $HClO_4$ extracts of rat brains. The ^{1}H NMR studies of the extracts solutions in D_2O were performed using a Varian Inova-300 NMR spectrometer. The integral intensities of the metabolite signals were found to change during the irradiation cycle and after it. These changes are accompanied by the variations in the T1 relaxation times. N-acetylaspartate, Glycerophosphocholine, Phosphocholine, Choline, Creatine and Phosphocreatine, myoInositol and Taurine were analysed as potential markers of irradiation injury. [*Sol State Nucl Magn Reson*, 2004;25:53-65]

Sokół M, Flakus L. ***Metabolic changes in epileptic patients – 1H NMR in vivo study***,

Epilepsy is a disease of diversified etiology. Patients with epilepsy are estimated to constitute about 1% of whole population. Approximately 30% of them suffers intractable epilepsy which often results in major medical and social disability for the individuals affected. The standard surgical treatment of these patients is anterior temporal lobectomy. It is there necessary to identify accurately the seizure focus. Several methods are used: MRI, EEG, SPECT and neuropsychologic testing. Chemical Shift Spectroscopy (CSI) is also helpful in this task.
The aim of this work was to characterize the metabolic profile of the epileptic brain.
Ten patients with intractable temporal lobe epilepsy were chosen to participate in the study. Ages ranged from 10 to 25 years. All patients underwent MR imaging and spectroscopic examinations. The whole-body MRI/MRS system (Elscint 2T Prestige) operating at a field strength of 2T and a proton resonance frequency of 81.3 MHz was used equipped with the standard head coil. The spectra were acquired from the volumes of 1.5x1.5x1.5cm3 using a double-spin-echo PRESS sequence with the following parameters: TR=1500 ms, TE=35 ms, 50 acquisitions.
Our study shows decrease in N-acetyl aspartate and strong disturbances in the glutamine and glutamate levels. [*Phys Med* 2004;20:21-24]

Cichoń A, Matulewicz Ł, Sokół M, Rutkowski T, ***Application of 1H MRS in brain metabolism studies in case of lung cancer***

Brain metabolism before and after a chest irradiation because of lung cancer was assessed by proton magnetic resonance spectroscopy in vivo. The aim of this work was to study the brain biochemical status of patients with lung cancer and to examine the possibility of early detection of metastatic brain tumours.
18 patients (divided into two age groups: 45-60 – 8 cases, and 60-74 – 10 cases) were examined using single-voxel 1H MRS (PRESS, TR=1500 ms, TE=35 ms, 50 Acq).
For the both studied groups the metabolic patterns were disturbed and similar. The NAA/tCr ratio was observed to be markedly reduced. The pronounced alternations of the metabolite levels are observed also for alanine and lipids. The latter two metabolites seem to be the metastase markers. [*Phys Med* 2004;20:24-27]

Matulewicz Ł, Cichoń A, Mych M, Sokół M. ***Mathematical model of brain response on irradiation***

The preliminary results on the mathematical model application for evaluating brain response to irradiation are shown. The model was constructed basing upon the 1H NMR in vivo data and tested on the high resolution 1H NMR in vitro results obtained in the animal model studies. The proposed mathematical model is found to describe properly the changes of the experimental signal intensity vs. dose for NAA and creatine, however it fails in case of cholines. [*Phys Med* 2004;20:81-86]

Matulewicz Ł, Cichoń A, Sokół M. ***A new potentially useful quantitative parameter of the local metabolic brain state after irradiation calculated from 1H MRS spectra***

Purpose: To monitor patients after radiotherapy and to check/found a reliable marker correlating over time.
Methods: Three patients were monitored by means of 1H MRS before and during 16/20/29 months after radiation therapy.
Results: The lipid CH2/CH3 ratio revealed a linear negative correlation vs. time after irradiation for the patients with no tumor recurrence. In case of tumor recurrence increase of the lipid CH2/CH3 value was observed.
Conclusion: The analyses of the chemical shifts of the lipid resonances provided a simple yet powerful means for differentiation of the recurrent tumor from radiation induced alterations. [*MAGMA*, 2004;17(Suppl 1)

Sokół M, Maciejowski M, Cichoń A, Gibas M. ***Application of in vivo and in vitro 1H NMR in multiple sclerosis studies***

Purpose: Numerous magnetic resonance spectroscopic studies have demonstrated the presence of metabolic abnormalities in CNS in patients with multiple sclerosis (MS). In particular, the decrease in the N-acetyl-L-aspartate (NAA) has been reported. The aim of this study was to get a better insight into the metabolic changes in relapsing-remitting RRMS patients as well as in those with clinically isolated syndromes (CIS). In vitro high-resolution 1H NMR of the cerebrospinal fluid (CSF) was chosen as a complement to in vivo proton cerebral MRS.

Subjects and Methods: Single-voxel proton magnetic resonance spectroscopy was performed in 16 patients (12 RR, 4 CIS) with clinically diagnosed MS, and 18 controls. Spectra from 3.4 ml voxels located in frontal lobes and periventricular regions (4 spectra/patient) were acquired with a PRESS sequence (1500ms/35ms/100, TR/TE/acquisitions) and analysed using automatic fitting procedure resolving overlapping peaks [2]. Signals due to NAA, cholines (Cho), total creatine (tCr), glucose (Glc), glutamate+glutamine (Glx), myoInositol (mI), taurine (Tau), lipids+lactate (Lip+Lac) were analysed.

High-resolution NMR measurements were done for 10 CSF samples (6 RR, 4 normal). A Varian Inova-300 multinuclear pulsed NMR spectrometer operating at the 1H resonance frequency of 300 MHz was used. CSF was lyophilised and resolved in D2O.

Results: Table 1 shows the statistically important metabolite ratios in CIS and RRMS patients as compared to the control group as well as those which are statistically different in a direct comparison.

p<0.005	Localization	NAA/tCr	Cho/tCr	Cho/NAA	Glc/tCr	Tau/tCr	(Lip+Lac)/ tCr
CIS vs. normal	F			+	+	+	
	P			+	+	+	
RRMS vs. normal	F	+	+	+	+	+	+
	P	+		+	+	+	+
RRMS vs. CIS	F			+			
	P	+	+				

As reveals from the high-resolution NMR studies of CSF, for RRMS patients the Glc level is higher than for the controls ($p<0.05$), whereas the mI and Glx integral intensities are the same as the normal values, thus confirming the observations from the in vivo spectra.

Conclusion: The biochemical status analysis in MS should not be restricted to NAA, Cho and Cr. Other metabolites, as glucose and taurine, seem to be sensitive and early indicators of metabolic disturbances.

Combining the in vivo and in vitro 1H NMR methods may give a better definition of the mechanisms associated with MS. [*MAGMA*, 2004;17(1)]

Tarasów E, Walecki J, Kubas B, Czernicki Z, Lewko J, Podgórski J, Sokół M. ***The importance of 1H MRS examination in the assessment of peritumor infiltration in patients with cerebral glioma***

Determination of the borders of the tumors plays crucial role to planning adequate therapy and enables prognosis. Diagnostic imaging methods do not allow to evaluate the boundaries of the infiltrate precisely. In this study we try to evaluate metabolite ratios in 1H MRS spectra to assess tumor spreading in border zone and to determine diagnostic values these parameters in predicting follow up. The objective of the study was to assess the usefulness of 1H MRS in the evaluation of the extent of the infiltrate around the apparently unchanged tumor area.

Material/Methods: The study group consisted of 64 patients with cerebral glioma. In all patients MR and single-voxel MR spectroscopy were performed. MRS spectra were obtained from the solid part of the tumor and from the VOI's placed in the borders of the tumor.

Results: The analysis of the metabolite ratios in the groups of patients with recurrent malignant lesions and in patients without signs of recurrent lesions revealed statistically significant differences for Lip/Cr and Lac/Cr ratios in the consequent voxels in peritumoral zone.

Conclusions: Our results indicate that analyse of the spectra and metabolite ratios in uncertain zone enables to depict neoplasmatical infiltration in morphologically unchanged tissue. The demonstration of metabolic changes in the uncertain zone can be potentially used for the selection of an appropriate operative treatment and radiotherapy because of ability to discriminate the group of patients with a higher risk of a recurrent lesion. [*Pol J Radiol*, 2004; 69(2): 16-23]

Rutkowski T, Cichoń A, Sokół M, Zajusz A. ***Alternation of brain tissue metabolism assessed by 1H-MRS in patients treated for NSCLC***

Aim: To assess the brain tissue metabolism using 1H-MRS in patients treated for NSCLC.
Material and Methods: The studied group consisted of 34 patients with NSCLC treated with radical intention in the Center of Oncology Maria Skłodowska-Curie Memorial Institute in Gliwice. 1H-MRS single voxel spectra were acquired from frontal (FL) and occipital lobes (OL). The control group consisted of 30 healthy volunteeres (CG).
Results: NAA/Cr was significantly lower, Cho/Cr and Cho/NAA were significantly elevated particularly in FL. Considerable elevated Lac/NAA and Lip/NAA were observed in FL and OL.
Conclusion: Brain metabolism is significantly changed in patients with NSCLC. At the time being is difficult to predict the practical value of proposed diagnostic approach. Thus, further study is needed to establish mechanism underlying observed alternation. This is crucial particularly for proper prevention and treatment. [*Rec Adv Res Upd Med* 2004;5(1):17-27]

Rutkowski T, Tarnawski R, Sokól M, Maciejewski B. ***1H-MR spectroscopy of normal brain tissue before and after postoperative radiotherapy because of primary brain tumors***

Purpose: Brain metabolism after surgery and postoperative radiotherapy (pRT) because of primary brain tumors was assessed by proton magnetic resonance spectroscopy (1H-MRS) in vivo. The study was designed to reveal the impact of pRT on normal brain tissue metabolism, which may potentially help in delineating the target volumes for reirradiated patients.
Methods and Materials: Spectra of 43 patients ages 16–63 years treated with pRT for primary glial tumors in the Center of Oncology Maria Curie Memorial Institute Branch in Gliwice were analyzed. The control group consisted of spectra acquired for 30 healthy volunteers. All patients were treated with 3D conformal techniques using 6–20 MV photons to total doses of 60 Gy. Spectra were acquired from the control region before pRT and from three uninvolved regions 9–12 months after the end of pRT. Voxels were located in the region of low (<6 Gy), medium (29–39 Gy), and high radiation dose (□60 Gy). Relative intensities of the signals relating to N-acetyl-aspartate (NAA), choline-based compounds, creatine and phosphocreatine (Cr), mio-Inositol, lactate, and lipids were obtained.
Results: The spectra of "normal brain" taken 9 months after pRT are significantly different from those obtained for control volunteers and from the spectra acquired before radiotherapy. The lactate and lipids signals are very strong; however, they are not correlated with absorbed dose. NAA/Cr ratios are significantly lower than before radiotherapy even for the low-dose regions. Differences increase with radiation dose: the NAA/Cr ratio is significantly lower for the regions of brain receiving a high dose of radiation than for the low-dose areas.
Conclusion: Combined treatment of primary brain tumors (surgery & postoperative radiotherapy) causes alteration of brain metabolism, even in regions of the brain far from the postoperative tumor bed and receiving relatively low total doses of radiation. Single voxel MRS spectroscopy in vivo cannot help in delineating target volumes for secondary irradiation. [*Int J Radiat Oncol Biol Phys* 2003;56(5):1381-9]

Walecki, J, Tarasów E, Kubas B, Czernicki Z, Lewko J, Podgórski J, Sokół M, Grieb P, ***Hydrogen-1 MR spectroscopy of the peritumoral zone in patients with cerebral glioma: assessment of the value of the method***

RATIONALE AND OBJECTIVES: The determination of tumor boundaries, especially in high-grade glioma, is critically important for the proper planning of treatment, but the standard diagnostic imaging methods do not enable precise delimitation of the extent of tumor cell infiltration into the surrounding tissue. The objective of this study was to assess the usefulness of hydrogen-1 (H-1) magnetic resonance (MR) spectroscopy for determining the extent of gliomatous infiltrate in the "uncertain zone'--the peritumoral area that appears unchanged on standard diagnostic MR images.
MATERIALS AND METHODS: The study group consisted of 64 patients with cerebral glioma scheduled for tumor resection and subsequent radiation therapy. All patients were examined prior to resection with MR imaging and MR spectroscopy. MR spectra were obtained from examination of the solid part of the tumor and from two peritumoral volumes of interest located approximately along the axis of surgical access to the tumor. MR spectra obtained from a group of 32 healthy volunteers were used as control data.
RESULTS: Analysis of the consequent voxels in the peritumoral zone revealed statistically significant differences in lipid/creatine and lactate/creatine metabolite ratios between patient subgroups with recurrent malignant lesions and without recurrent lesions. Significant differences also were found between the patient group and the control group in most metabolite ratios assessed.
CONCLUSION: H-1 MR spectroscopic demonstration of metabolic changes in the peritumoral zone can guide treatment for cerebral glioma, enabling the physician to identify patients who have a high risk of recurrence. [*Acad Radiol* 2003;10:145-153]

Tarnawski R., Sokół M., Pieniążek P., Maciejewski B., Walecki J., Miszczyk L., Krupska T.

1H-MRS in vivo predicts the early treatment outcome of postoperative radiotherapy for malignant gliomas

PURPOSE: To analyze prospectively the prognostic significance of 1H magnetic resonance spectroscopy (MRS) in vivo recorded from the tumor bed of patients after surgery for malignant glioma.

METHODS AND MATERIALS: Fifty-one patients aged 20-68 years were examined using a MRI/MRS system (Elscint 2T Prestige). Of the 51 patients, 33 had Grade 3 gliomas and 18 had glioblastomas. MRI-localized 1HMR spectra were acquired using a single-voxel, double-spin-echo sequence. Relative intensities of the signals (choline, creatine [Cr] N-acetyl aspartate [NAA], myo-inositol, lactate, and lipids) were obtained by numeric integration of fitted signals. Two voxels were examined, one located at the tumor bed and the second distant to the tumor bed. All patients were irradiated to 60 Gy using three-dimensional conformal noncoplanar techniques to 60 Gy.

RESULTS: MRS in vivo in patients after brain tumor surgery revealed a statistically significant decrease in the NAA/Cr ratio and increases in the choline/creatine (Cr), choline/NAA, and myo-inosytol/Cr ratios. The intensive signals of lactate and lipids appeared in spectrum. Survival correlated strongly with tumor grade and patient age but the strongest prognostic factor was the lactate/NAA ratio. For lactate/NAA values >2.0 (intensive lactate signal) the 1-year survival rate was 20%, and for lactate/NAA values <2.0, the 1-year survival rate was 85%.

CONCLUSION: A new diagnostic tool demonstrated ability to distinguish between patients with a favorable prognosis and those who will die within 1 year. [*Int. J. Rad. Oncol. Biol. Phys*. 2002:52(5)]

Department of Molecular Biology

Research currently pursued at this Department is concerned with devising novel and specific strategies of destroying neoplastic tumors. Investigations have been focusing in particular on the application of antiangiogenic proteins and antivascular drugs in combination with chemo- and radiotherapy modalities.

The following research topics have been of particular interest to this group:

1. New antivascular drugs recognizing $\alpha_V\beta_3$ integrins and VEGF receptors present on endothelial cell surface of tumor vessels

2. Recombinant antiangiogenic proteins isolated from *E. coli*

3. Construction of novel liposomal and other cationic polymeric carriers enabling transport of drugs and nucleic acids

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Selected Papers:

Szala S. ***Two-domain vascular disruptive agents in cancer therapy.***
The two-domain vascular drug constructs are selective anti-cancer agents capable of specific targeting and subsequent elimination of endothelial cells lining tumor blood vessels. The destruction of existing vasculature within tumor tissue causes insufficient oxygenation of adjacent neoplastic cells and their necrotic death. The recognition (cognitive) domain of the vascular disruptive agents is responsible for recognizing markers specific for endothelial cells. This domain can be formed by variable regions of antibodies or by suitable ligands (such as those binding various integrin or growth factor receptors). The effector domain, in turn, can be constructed from proteins participating in blood clotting process, as well as from toxins, cytokines, radioactive isotopes or pro-apoptotic factors. This article outlines issues important for constructing such two-domain vascular disruptive agents and emphasizes the modularity of their assembly. Several pharmacokinetic and pharmacodynamic properties of these novel agents are discussed. Compared to known cytostatic substances exerting anti-angiogenic effects, such vascular disruptive agents can be much more effective as cytotoxic agents, especially in combination with proven anti-cancer drugs. [*Curr Cancer Drug Targets*. **4** (2004): 501-9].

Sochanik A, Cichon T, Makselon M, Stróżyk M, Smolarczyk R, Jazowiecka-Rakus J, Szala S. ***In vivo gene transfer using cetylated polyethylenimine.***
This report describes gene transfer in vitro as well as in vivo using cetylated low-molecular mass (600 Da) polyethylenimine (28% of amine groups substituted with cetyl moieties), termed CT-PEI. This compound is hydrophobic and has to be incorporated into liposomes in order to be suitable for gene transfer studies. Serum-induced plasmid DNA degradation assay demonstrated that CT-PEI-containing liposomal carriers could protect complexed DNA (probably via condensation). In vitro luciferase gene expression achieved using medium supplemented with 10% serum was comparable to that achieved in serum-reduced medium and was highest for CT-PEI/cholesterol liposomes, followed by CT-PEI/dioleoylphosphatidylcholine liposomes and PEI 600 Da (uncetylated) carrier. In vivo systemic transfer into mice was most efficient when liposome formulations contained CT-PEI and cholesterol. Higher luciferase expression was then observed in lungs than in liver. In conclusion: liposomes containing cetylated polyethylenimine and cholesterol are a suitable vehicle for investigating systemic plasmid DNA transfer into lungs. [Acta Biochim Polon. **51** (2004): 693-702]

Jazowiecka-Rakus J, Szala S. ***Antitumour activity of Salmonella typhimurium VNP20047 in B16(F10) murine melanoma model.***
A tumour therapy is proposed based on attenuated Salmonella typhimurium VNP20047 expressing the Escherichia coli cytosine deaminase gene. VNP20047 was administered intravenously to B16(F10) melanoma-bearing C57BL/6 mice. VNP20047 proliferated within tumours and livers regardless of the initial inoculum dose. After 10 days the number of bacteria increased in livers up to 4.2 x 10(6) cfu/g and decreased in tumours down to 5.9 x 10(6) cfu/g. VNP20047 at 1 x 10(5) cfu/mouse, when combined with 5-fluorocytosine, inhibited tumour growth by 85% without prolonging animal survival. Histology studies revealed severe lesions in tumours and livers. These data suggest that S. typhimurium VNP20047 induced inflammatory responses, even though the strain was attenuated. [*Acta Biochim Polon*. **51** (2004): 851-6].

Dąbrowska A, Szary J, Kowalczuk M, Szala S, Ugorski M. ***CEA-negative glioblastoma and melanoma cells are sensitive to cytosine deaminase/5-fluorocytosine therapy directed by the carcinoembryonic antigen promoter.***
Recent studies have suggested that carcinoembryonic antigen (CEA)-promoter sequences are active only in CEA-positive cells, filing in the criteria for tumor specific targeting of suicide genes. However, the present study on gene therapy of colon cancer and cell-specificity of CEA promoter, provide evidence that CEA-positive and CEA-negative cells transfected with E. coli cytosine deaminase (CD) gene under the control of CEA promotor sequence are sensitive to enzyme/pro-drug therapy with 5-fluorocytosine (5-FC). Individual clones derived from the CEA-negative cell lines: melanoma Hs294T and glioblastoma T98G after transfection with CD differed profoundly in their sensitivity to 5-FC. The IC50 values for several clones of the CEA-negative cells were almost the same as for CEA-positive colon cancer cells. Such 5-FC-sensitive clones derived from the population of CEA-negative cells, present even in small number, because of the very effective bystender effect of this enzyme/pro-drug system can cause severe problems during therapy by efficiently killing surrounding normal cells. Safety is the major issue in gene therapy. Our data suggest that the safety of gene-directed enzyme pro-drug therapy (GDEPT) with CEA promoter driven expression of therapeutic genes is not so obvious as it has originally been claimed. [*Acta Biochim Polon*. **51** (2004): 723-32.]

Markowska J, Szala S. ***Inhibitors of angiogenesis in therapy of ovarian cancers.*** The capacity to induce growth of blood vessels represents one of the phenotypic traits of neoplastic cells. Several preclinical studies prove that the inhibition of growth of peri-neoplastic blood vesssels leads to restricted growth of primary tumours and of metastases. Nevertheless, clinical studies indicate that angiogenesis inhibitors are not such effective drugs as might be expected on the basis of studies conducted on animals. In this article we would like to draw the readers' attention to divergencies between preclinical and clinical results, in particular to those related to ovarian cancers. In the treatment of ovarian cancers, angiogenesis inhibitors combined with other drugs may prove to represent a relatively effective therapeutic approach. [*Eur J Gynaecol Oncol*. **25** (2004): 562-7]

Cichoń T., Jamroży L., Głogowska J., Missol-Kolka E. Szala S.: ***Electrotransfer of gene encoding endostatin into normal and neoplastic mouse tissues: Inhibition of primary tumor growth and metastatic spread.***
Electroporation-mediated gene transfer relies upon direct delivery of plasmids into cells permeabilized by electric fields, a method more efficient than transfer using nonviral vectors, although neither approaches the transfer efficiency of viral vectors. Here we studied electrotransfer of a gene encoding an angiogenesis inhibotor (endostatin) into primary tumors and muscle tissues which would serve as a site of synthesis and secretion into the bloodstream of a therapeutic antimetastatic protein with systemic effects. Optimum electroporation conditions were first established to maximize the expression of the reporter gene transferred into Renca kidney carcinoma, B16(F10) murine melanoma or skeletal muscle tissues. In neoplastic tissues electrotransfer of plasmid DNA was far more efficient than electroporation with lipoplexes. We then studied electrotransfer of plasmid DNA carrying the endostatin gene into pre-established experimental Renca tumors. A significant inhibition of tumor growth was observed in animals electroporated with this construct. This study clearly shows that electroporation may be used to efficiently transfer antiangiogenic genes into both normal and neoplastic tissues. [*Cancer Gene Therapy* **9** (2002): 771-777]

Zemlińska B, Sochanik A., Missol-Kolka E., Szala S.: ***Various cationic carriers for in vitro transfection of tumor and endothelial cell lines.***
We compared the efficiency of in vitro DNA transfer ibto selected tumor and endothelial cell lines using complexes of plasmid DNA and cationic carriers DDAB/DOPE, DC-Chol/DOPE, Arg-Chol/DOPE,Gly-Chol/DOPE, Arg-Gly-Chol/DOPE, BGTC/DOPE and PEI. The best carriers for transfecting the majority of tested cell lines at optimized carrier-to-DNA weight ratios were PEI and BGTC/DOPE. [*Acta Biochim. Polon.* **49** (2002) 285-290]

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Current research interest:

Laboratory of Cancer Genetics is engaged in the studies of the biology of ovarian and breast cancer using microarray technique. We study the molecular properties of the tumors arising on the basis of hereditary mutation in BRCA genes, as well as the differences in gene expression profiles between chemo-sensitive versus chemo-resistant cancer cases (different types of chemotherapy). We also investigate the molecular difference between certain histopatological types of these tumors. The group also search for mutation in the chaperon HSC70 protein in NSCLC. Recently the analysis of SNP and dinucleotide repeats in estrogen receptor genes α and β, and trinucleotide repeats in androgen receptor gene in the carriers of germline mutations in BRCA1/2 gene diagnosed with breast or ovarian cancer versus non carriers and healthy persons has been carried on.

Laboratory of Immunohistochemistry is engaged in studies of expression pattern of stress proteins (HSP25, HSP70) in rat liver after treatment with some intoxicants (thioacetamide, D-galactosamine, or allyl alcohol) alone or in combination with certain antiinflammatory drugs in order to establish the role of HSP25 in inflammatory processes. The group is also interested in the expression of some cell cycle proteins and stress proteins HSP70 and HSP27 in non small cell lung cancer.

Laboratory of Molecular Mechanisms of Carcinogenesis. The previous and current research topics include: analysis of DNA mutations in cancer cells, studies on polymorphisms within DNA repair and stress-response genes in relation to the lung cancer risk in Polish population, functional studies on polymorphic alleles of DNA repair and stress-response genes, exploring functional interactions of DNA repair proteins (nucleotide excision repair proteins and RecQ helicases), regulation of cellular senescence.

Laboratory of Stress Genes is involved in structural and functional analysis of the rat heat shock 70 gene family. We discovered a new gene (called the hst70) which is involved in the differentiation of spermatogenic cells. Part of the group is involved in the study of the regulation of the expression of that gene using a model of transgenic mice. The group is also studying mechanisms of seminiferous epithelium degeneration after elevation of the testes temperature as well as the activity of the testis-specific heat shock protein (hst70) gene in somatic cells of adult mice and during embryogenesis. Another project concentrates on the cytoprotective role of heat shock proteins in cell survival and integration of mitotic spindle. Also the project, the aim of which is to investigate the HSPs as potential autologous anticancer vaccines is in progress. The group is also involved in the study of drug delivery systems and current interest is concentrated on cytotoxic properties of drugs conjugated with oligomers of 3-hydroxybutyric acid.

Selected Papers:

Pack S.D., Alper O.M., Stromberg K., Augustus M., Ozdemirli M., Miermont A.M., Klus G., Rusin M., Slack R., Hacker N.F., Ried T., Szallasi Z., Alper O.: ***Simultaneous suppression of epidermal growth factor receptor and c-erbB-2 reverses aneuploidy and malignant phenotype of a human ovarian carcinoma cell line.***
Coexpression of epidermal growth factor receptor (EGFR) and c-erbB-2 in 47-68% of ovarian cancer cells indicate their strong association with tumor formation. We examined the effects of simultaneous antisense- or immunosuppression of EGFR and c-erbB-2 expression on the invasive phenotype, aneuploidy, and genotype of cultured human ovarian carcinoma cells (NIH:OVCAR-8). We report here that suppression of both EGFR and c-erbB-2 results in regression of aneuploidy and genomic imbalances in NIH:OVCAR-8 cells, restores a more normal phenotype, and results in a more normal gene expression profile. Combined with cytogenetic analysis, our data demonstrate that the regression of aneuploidy is due to the selective apoptosis of double antisense transfected cells with highly abnormal karyotype. [*Cancer Research* **64** (2004), 789-94].

Ścieglińska D., Vydra N., Krawczyk Z., Widlak W.: ***Location of promoter elements necessary and sufficient to direct testis-specific expression of the Hst70/Hsp70.2 gene.***
The rat Hst70 gene and its mouse counterpart Hsp70.2 are expressed specifically in pachytene primary spermatocytes and spermatids. Here we demonstrate that a 165 bp fragment of the Hst70 gene promoter, containing the T1 transcription start site region, entire exon 1 and 42 bp 5' region of the intron, is sufficient to drive testis-specific expression of the chloramphenicol acetyltransferase reporter gene in transgenic mice with the same developmentally regulated pattern as the endogenous Hsp70.2 gene. We show further that high-level tissue-specific gene expression requires additional sequences localized upstream of the T2 transcription start site. Electrophoretic mobility-shift assay analysis revealed that only testes of juvenile rats, when Hst70 gene expression is repressed, contain proteins that specifically bind to the Oct (octamer) sequence localized directly downstream of the T1 site. [*Biochemical Journal* **379** (2004), 739-47].

Försti A., Angelini S., Festa F., Sanyal S., Zhang Z., Grzybowska E., Pamuła J., Pękala W., Zientek H., Hemminki K., Kumar R.: ***Single nucleotide polymorphisms in breast cancer.***
A limited number of genes have been identified that explain heritable risks of breast cancer (BC). We searched for low-penetrant genes in an association study using two populations: 223 Finnish unselected patients and 172 Polish familial cases, both with locally collected healthy controls. Candidate genes included DNA repair genes, methylenetetrahydrofolate reductase (MTHFR) and cyclin D1 genes. The frequencies for single nucleotide polymorphisms (SNPs) were measured in the following genes: NBS1, XPC, XPD, XRCC1, XRCC3, MTHFR, and cyclin D1. Odds ratios (ORs) were calculated to the wild-type genotype. The positive findings in the Finnish series were repeated in the Polish series. Significant findings among Finns were associations to XPC exon 15, XPD exon 10 and XRCC3 exon 7, the latter of borderline significance. None of these results could be repeated in the Polish series. The XPC result among Finns was probably an artifact of the control group deviating from the Hardy-Weinberg Equilibrium (HWE). The attempt to repeat the result for the XPD polymorphism among Poles was probably not valid because the control group deviated from the HWE. We conclude that within statistical power of the present study, none of the tested polymorphisms associated with BC, with the probable exception of XPD. [*Oncology Reports* **11** (2004), 917-22].

Widlak W.,Widlak P.: ***MAR/SAR elements flank the rat hst70 gene transcription unit.***
The rat hst70 gene is specifically expressed in spermatocytes and spermatids. This tissue-specific expression of the gene is primarily mediated through cis-acting elements located within the 0.4 kb segment upstream of the coding region, including two transcription initiation sites. Here, we study the 5' and 3' distal elements flanking the hst70 gene and find that they possess structural motifs characteristic of MAR/SAR elements, and exhibit enhanced affinities for nuclear matrix binding in vitro. Such elements bind efficiently to matrices from either the testis or the liver, i.e. tissues where the gene is either fully active or repressed, although one subfragment in the 5' region was identified as exhibiting testis-specific interactions. Surprisingly, the activity of the CAT reporter gene was repressed in testis-transient transfection assays when the hst70 promoter sequences were extended into the 5' MAR/SAR. [*Cellular & Molecular Biology Letters* **9** (2004), 123-33]

Górski B., Jakubowska A., Huzarski T., Byrski T., Gronwald J., Grzybowska E., Mackiewicz A., Stawicka M., Bębenek M., Sorokin D., Fiszer-Maliszewska L., Haus O., Janiszewska H., Niepsuj S., Góźdź S., Zaremba L., Posmyk M., Plużańska M., Kilar E., Czudowska D., Waśko B., Miturski R., Kowalczyk J.R., Urbański K., Szwiec M., Koc J., Debniak B., Rozmiarek A., Dębniak T., Cybulski C., Kowalska E., Tołoczko-Grabarek A., Zajaczek S., Menkiszak J., Mędrek K., Masojć B., Mierzejewski M., Narod S.A., Lubiński J.: ***A high proportion of founder BRCA1 mutations in Polish breast cancer families.***

Three mutations in BRCA1 (5382insC, C61G and 4153delA) are common in Poland and account for the majority of mutations identified to date in Polish breast and breast-ovarian cancer families. It is not known, however, to what extent these 3 founder mutations account for all of the BRCA mutations distributed throughout the country. This question has important implications for health policy and the design of epidemiologic studies. To establish the relative contributions of founder and nonfounder BRCA mutations, we established the entire spectrum of BRCA1 and BRCA2 mutations in a large set of breast-ovarian cancer families with origins in all regions of Poland. We sequenced the entire coding regions of the BRCA1 and BRCA2 genes in 100 Polish families with 3 or more cases of breast cancer and in 100 families with cases of both breast and ovarian cancer. A mutation in BRCA1 or BRCA2 was detected in 66% of breast cancer families and in 63% of breast-ovarian cancer families. Of 129 mutations, 122 (94.6%) were in BRCA1 and 7 (5.4%) were in BRCA2. Of the 122 families with BRCA1 mutations, 119 (97.5%) had a recurrent mutation (i.e., one that was seen in at least 2 families). In particular, 111 families (91.0%) carried one of the 3 common founder mutations. The mutation spectrum was not different between families with and without ovarian cancer. These findings suggest that a rapid and inexpensive assay directed at identifying the 3 common founder mutations will have a sensitivity of 86% compared to a much more costly and labor-intensive full-sequence analysis of both genes. This rapid test will facilitate large-scale national epidemiologic and clinical studies of hereditary breast cancer, potentially including studies of chemoprevention. [*International Journal of Cancer* **110** (2004), 683-86].

Smits K.M., Benhamou S., Garte S., Weijenberg M.P., Alamanos Y., Ambrosone C., Autrup H., Autrup J.L., Baranova H., Bathum L., Boffetta P., Bouchardy C., Brockmoller J., Butkiewicz D., Cascorbi I., Clapper M.L., Coutelle C., Daly A.K., Muzi G., Dolzan V., Duzhak T.G., Farker K., Golka K., Haugen A., Hein D.W., Hildesheim A., Hirvonen A., Hsieh L.L., Ingelman-Sundberg M., Kalina I., Kang D., Katoh T., Kihara M., Ono-Kihara M., Kim H., Kiyohara C., Kremers P., Lazarus P., Le Marchand L., Lechner M.C., London S., Manni J.J., Maugard C.M., Morgan G.J., Morita S., Nazar-Stewart V., Kristensen V.N., Oda Y., Parl F.F., Peters W.H., Rannug A., Rebbeck T., Pinto L.F., Risch A., Romkes M., Salagovic J., Schoket B., Seidegard J., Shields P.G., Sim E., Sinnett D., Strange R.C., Stucker I., Sugimura H., To-Figueras J., Vineis P., Yu M.C., Zheng W., Pedotti P., Taioli E.: ***Association of metabolic gene polymorphisms with tobacco consumption in healthy controls.***

Polymorphisms in genes that encode for metabolic enzymes have been associated with variations in enzyme activity between individuals. Such variations could be associated with differences in individual exposure to carcinogens that are metabolized by these genes. In this study, we examine the association between polymorphisms in several metabolic genes and the consumption of tobacco in a large sample of healthy individuals. The database of the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens was used. All the individuals who were controls from the case-control studies included in the data set with information on smoking habits and on genetic polymorphisms were selected (n = 20938). Sufficient information was available on the following genes that are involved in the metabolism of tobacco smoke constituents: CYP1A1, GSTM1, GSTT1, NAT2 and GSTP1. None of the tested genes was clearly associated with smoking behavior. Information on smoking dose, available for a subset of subjects, showed no effect of metabolic gene polymorphisms on the amount of smoking. No association between polymorphisms in the genes studied and tobacco consumption was observed; therefore, no effect of these genes on smoking behavior should be expected. [*International Journal of Cancer* **110** (2004) 266-70].

Piddubnyak V., Kurcok P., Matuszowicz A., Głowala M., Fiszer-Kierzkowska A., Jedlinski Z., Juzwa M., Krawczyk Z.: ***Oligo-3-hydroxybutyrates as potential carriers for drug delivery.***

In the present paper we describe the synthesis and toxicity studies of well-defined tailor made oligo-[R,S]-3-hydroxybutyrates (OHBs). The results indicate potential applicability of these nano-polymers as drug delivery

carriers. Several OHBs of number average molecular weight (M(n)) ranging from 800 to 2400 have been synthesized and tested on transformed hamster V79 fibroblasts and murine melanoma B16(F10) cells using the 3-[4,5-dimethylthiazol-2-yl]2,5-diphenyltetrazolium bromide (MTT) based drug resistance and clonogenic survival assays. We show that 96-h incubation of cells with 1-9 microg/ml of OHBs did not affect cell viability. Incubation of OHBs with rat hepatoma FTO-2B cells stably transfected with chloramphenicol acetyltransferase (CAT) gene ligated to heat-inducible hsp70i gene promoter demonstrated that OHBs did not induce cellular stress response. Finally, we demonstrate that doxorubicin conjugated with OHB is effectively taken up by murine melanoma B16(F10) cells in vitro and localizes in the cytoplasm. These data show for the first time that tailor-made biodegradable and biocompatible oligomers of 3-hydroxybutyric acid can be taken into consideration as effective, non-toxic vectors for delivery of drugs in a conjugated form. [*Biomaterials* **25** (2004) 5271-79].

Jin Q., Hemminki K., Grzybowska E., Klaes R., Soderberg M., Zientek H., Rogozinska-Szczepka J., Utracka-Hutka B., Pamuła J., Pękala W., Försti A.: ***Polymorphisms and haplotype structures in genes for transforming growth factor β1 and its receptors in familial and unselected breast cancers.***

Alterations in TGF-beta signaling appear to be associated with an altered risk of developing cancer, including breast cancer. We carried out a case-control study on 8 polymorphisms, including 5 in the *TGF-β1* gene (G-800A, C-509T, Leu^{10}→Pro, Arg^{25}→Pro and Thr^{263}→Ile), a polyalanine polymorphism (9A→6A) in the *TGF-βRI* gene and 2 (G-875A and A-364G) in the *TGF-βRII* gene, using samples from 2 different populations, Polish familial and Finnish unselected breast cancer cases, together with ethnically and geographically matched controls. Additionally, familial breast cancer cases with respective controls from Sweden and Germany were studied in the Leu^{10}→Pro polymorphism, making the total number of familial cases 659. Allele, genotype and haplotype analysis on the *TGF-β1* gene as well as an analysis of the combinations of genotypes of the *TGF-β1* and its receptor genes in each individual were performed. Population differences in the allele and genotype distributions were found from 5 of the polymorphisms and 3 common haplotypes from the *TGF-β1* gene between the Finnish and other populations. However, no statistically significant difference between the breast cancer and healthy control groups was found for any of the 8 polymorphisms nor did the haplotype or genotype combination analysis reach statistical significance. Thus, none of the studied polymorphisms from the *TGF-β1* and its receptor genes was found to influence significantly susceptibility to breast cancer. The possible contribution of 6A/6A homozygosity in the *TGF-βRI* gene to breast cancer needs to be confirmed in an independent study. [*International Journal of Cancer* **112** (2004), 94-99].

Wagner K., Hemminki K., Grzybowska E., Klaes R., Butkiewicz D., Pamuła J., Pękala W., Zientek H., Mielżyńska D., Siwińska E., Försti A.: ***The insulin-like growth factor-1 pathway mediator genes: SHC1 Met300Val shows a protective effect in breast cancer.***

The insulin-like growth factor 1 (IGF-1) pathway plays an important role in regulating cell proliferation, differentiation and apoptosis. IRS1, IRS2 and SHC1 are the key mediators for the downstream pathway processes. Genetic variation within these genes may lead to altered signalling. We screened IRS1, IRS2 and SHC1 for published coding region polymorphisms and choose five of them, IRS1 Ala804Ala and Gly972Arg, IRS2 Cys816Cys and Gly1057Asp and SHC1 Met300Val, for further analysis. We studied the association of the polymorphisms with breast cancer risk using a case-control design with Polish familial breast cancer cases and respective controls. For the polymorphisms in IRS1 and IRS2 no differences in the allele, genotype or haplotype distributions could be detected between the case and control subjects. Carriers of the variant allele of the SHC1 polymorphism were at decreased risk of breast cancer (OR 0.54, 95% CI 0.32-0.90, P = 0.016). A non-significant trend for a protective effect of the SHC1 Val300 allele was also seen in an independent population consisting of German familial breast cancer cases and matched controls. The joint analysis after Mantel-Haenzsel adjustment of the two populations gave an OR of 0.62 (95% CI 0.41-0.93, P = 0.02) for the SHC1 Val300 carriers. A stronger effect was detected in women diagnosed below the age of 50 (OR 0.54, 95% CI 0.32-0.89, P = 0.01). A genotype combination analysis of the non-synonymous polymorphisms in the IRS1, IRS2 and SHC1 genes did not show any effect on breast cancer risk. [*Carcinogenesis* **25** (2004), 2473-2478].

Rogozińska-Szczepka J., Utracka-Hutka B., Grzybowska E., Mąka B., Nowicka E., Smok-Ragankiewicz A., Zientek H., Steffen J., Wojciechowska-Łącka A.: ***BRCA1 and BRCA2 mutations as prognostic factors in bilateral breast cancer patients.***

BACKGROUND: Incidence of primary bilateral breast cancer (BC) is rare and does not exceed 5%. BRCA1/2 mutation carriers diagnosed with breast cancer have a strong life time risk of developing contralateral breast

cancer (53% versus 2%). PATIENTS AND METHODS: A group of 108 patients with bilateral breast cancer, who reported at our Cancer Centres from 2000 to 2002, were subjected to genetic testing. Similarities and differences between BRCA1/2 carriers and non-carriers were analysed in terms of family history, pathology of tumour, age of diagnosis, developing contralateral BC and second primary cancer. RESULTS: BRCA1/2 mutations were detected in 32 of 108 patients. Family history of BC was identified in 46.9% of these patients compared with 22.4% of non-carriers (P <0.05). Synchronous BC was diagnosed significantly rarer [4 of 32 (12.5%)] in BRCA1/2 carriers than in the non-carrier group [26 of 76 (34.2%)]. In addition, patients with BRCA mutations were younger when they were diagnosed than non-carriers. BRCA1/2 carriers had a significantly higher incidence of medullary BC (13.6% versus 1.7%) and developed ovarian cancer significantly more frequently than non-carriers (12 of 32 and 1 of 72 patients, respectively). CONCLUSIONS: Patients with bilateral BC having BRCA mutations are significantly younger than non-carriers. They also have a significantly higher family history of BC and an increased risk of developing ovarian cancer. The differences in clinical aspects of BRCA carriers with bilateral BC should be considered in clinical management. [*Annals of Oncology* **15** (2004), 1373-1376].

Łobocka M.B., Rose D.J., Plunkett G. 3rd, Rusin M., Samojedny A., Lehnherr H., Yarmolinsky M.B., Blattner F.R.: ***Genome of bacteriophage P1.***

P1 is a bacteriophage of Escherichia coli and other enteric bacteria. It lysogenizes its hosts as a circular, low-copy-number plasmid. We have determined the complete nucleotide sequences of two strains of a P1 thermoinducible mutant, P1 c1-100. The P1 genome (93,601 bp) contains at least 117 genes, of which almost two-thirds had not been sequenced previously and 49 have no homologs in other organisms. Protein-coding genes occupy 92% of the genome and are organized in 45 operons, of which four are decisive for the choice between lysis and lysogeny. Four others ensure plasmid maintenance. The majority of the remaining 37 operons are involved in lytic development. Seventeen operons are transcribed from sigma(70) promoters directly controlled by the master phage repressor C1. Late operons are transcribed from promoters recognized by the E. coli RNA polymerase holoenzyme in the presence of the Lpa protein, the product of a C1-controlled P1 gene. Three species of P1-encoded tRNAs provide differential controls of translation, and a P1-encoded DNA methyltransferase with putative bifunctionality influences transcription, replication, and DNA packaging. The genome is particularly rich in Chi recombinogenic sites. The base content and distribution in P1 DNA indicate that replication of P1 from its plasmid origin had more impact on the base compositional asymmetries of the P1 genome than replication from the lytic origin of replication. [*Journal of Bacteriology* **186** (2004) 7032-7068].

Krześniak M., Butkiewicz D., Samojedny A., Chorąży M., Rusin M. ***Polymorphisms in TDG and MGMT genes – epidemiological and functional study in lung cancer patients from Poland.***

The genetic, functional polymorphisms of DNA repair genes are good candidates for cancer susceptibility markers. We studied genes (*MGMT* and *TDG*) coding for proteins removing small DNA adducts by direct repair (MGMT), or mispaired DNA bases by the base excision repair (TDG). The non-silent polymorphisms of *MGMT* (84:Phe, 143:Val, 178:Arg), *TDG* (199:Ser, 367:Met) and the functional *MGMT* enhancer polymorphism did not show any statistically significant association with lung cancer risk in our case-control analysis, but due to the relatively small number of individuals, the strong conclusions on the cancer risk association or lack thereof can not be made. Sequencing of the *TDG* cDNA has not revealed any novel polymorphism but an alternatively spliced mRNA with missing exon 2. Our search for polymorphisms within the promoter-enhancer region of *MGMT* revealed three novel sequence variants. The functional significance of the previously published *MGMT* enhancer polymorphism (1099C->T) was assessed. The less frequent sequence variant of the enhancer was associated with modest (16-64%), but statistically significant, increase of *MGMT* promoter-enhancer activity in the studied cell lines. This work points to the importance of studying the expression-regulating elements of genes, because they contain functional polymorphisms with potential for modulating risk of various diseases, including cancer. [*Annals of Human Genetics* **68** (2004): 300-12].

Rusin M., Zientek H., Krześniak M., Małusecka E., Zborek A., Krzyżowska-Gruca S., Butkiewicz D., Vaitiekunaite R., Lisowska K., Grzybowska E., Krawczyk Z.: ***Intronic polymorphism (1541-1542delGT) of the constitutive heat shock protein 70 gene has functional significance and shows evidence of association with lung cancer risk.***

Somatic mutations of 11q23.3-linked constitutive heat shock protein 70 gene (*HSPA8* alias *HSC70*) were detected by others in breast carcinomas. To examine whether intragenic, somatic mutations of *HSPA8* occur in lung carcinomas, we sequenced its exons 2 – 8, with adjacent intronic sequences, in a series of DNA samples

from non-small-cell lung cancers. Twenty one polymorphisms were detected, but no somatic mutation. However, we observed an association between the *HSC70* 1541-1542delGT genotype and immunohistochemical staining pattern of HSC70 protein. Tumors with the weak (+) HSC70 protein staining were more frequent in the carriers of the polymorphic 1541-1542delGT allele then in the homozygotes of the major allele (20% versus 6%, *P*=0.05 by Fisher's exact test). This statistically significant association prompted us to functionally test the polymorphism. The method developed by us for the functional evaluation of intronic sequence alterations showed that the *HSPA8* intron 2 with the deleted GT dinucleotide was associated with noticeable (approximately 20%) and statistically significant (p=0.005) reduction of the reporter gene activity. Our case-control analysis showed that the 1541-1542delGT heterozygous genotype was associated with significantly decreased risk for lung cancer (crude odds ratio = 0.44; 95% confidence interval: 0.23-0.84). To the best of our knowledge, this is the first report on the association between a polymorphism of a gene coding for the chaperone protein and lung cancer risk. Moreover, the simple method reported here, based on the dual-luciferase reporter assay system, can be useful for testing functional significance of polymorphisms located in introns of other genes. [*Molecular Carcinogenesis* **39 (**2004): 155-63].

Popanda O., Schattenberg T., Phong C.T., Butkiewicz D., Risch A., Edler L., Kayser K., Dienemann H., Schulz V., Drings P., Bartsch H., Schmezer P.: ***Specific combinations of DNA repair gene variants and increased risk for non-small cell lung cancer.***

Several polymorphisms in DNA repair genes have been reported to be associated with lung cancer risk including XPA (-4G/A), XPD (Lys751Gln and Asp312Asn), XRCC1 (Arg399Gln), APE1 (Asp148Glu) and XRCC3 (Thr241Met). As there is little information on the combined effects of these variants, polymorphisms were analyzed in a case-control study including 463 lung cancer cases [among them 204 adenocarcinoma and 212 squamous cell carcinoma (SCC)] and 460 tumor-free hospital controls. Odds ratios (OR) adjusted for age, gender, smoking and occupational exposure were calculated for the variants alone and combinations thereof. For homozygous individuals carrying the Glu variant of APE1, a protective effect was found (OR = 0.77, CI = 0.51-1.16). Individuals homozygous for the variants XPA (-4A) (OR = 1.53, CI = 0.94-2.5), XPD 751Gln (OR = 1.39, CI = 0.90-2.14) or XRCC3 241Met (OR = 1.29, CI = 0.85-1.98) showed a slightly higher risk for lung cancer overall. In the subgroup of adenocarcinoma cases, adjusted ORs were increased for individuals homozygous for XPA (-4A) (OR = 1.62, CI = 0.91-2.88) and XRCC3 241Met (OR = 1.65; CI = 0.99-2.75). When analyzing the combined effects of variant alleles, 54 patients and controls were identified that were homozygous for two or three of the potential risk alleles [i.e. the variants in nucleotide excision repair, XPA (-4A) and XPD 751Gln, and in homologous recombination, XRCC3-241Met]. ORs were significantly increased when all patients (OR = 2.37; CI = 1.26-4.48), patients with SCC (OR = 2.83; CI = 1.17-6.85) and with adenocarcinoma (OR = 3.05; CI = 1.49-6.23) were analyzed. Combinations of polymorphisms in genes involved in the same repair pathway (XPA + XPD or XRCC1 + APE1) affected lung cancer risk only in patients with SCC. These results indicate that lung cancer risk is only moderately increased by single DNA repair gene variants investigated but it is considerably enhanced by specific combinations of variant alleles. Analyses of additional DNA repair gene interactions in larger population-based studies are warranted for identification of high-risk subjects. [*Carcinogenesis* **25** (2004), 2433-2441].

Butkiewicz D., Popanda O., Risch A., Edler L., Dienemann H., Schulz V., Kayser K., Drings P., Bartsch H., Schmezer P.: ***Association between the risk for lung adenocarcinoma and a (-4) G-to-A polymorphism in the XPA gene.***

Polymorphisms of genes coding for DNA repair can affect lung cancer risk. A common single nucleotide (-4) G-to-A polymorphism was identified previously in the 5' untranslated region of the XPA gene. In a case-control study in European Caucasians, the influence of this polymorphism on primary lung cancer risk overall and according to histologic subtypes was investigated. Four hundred sixty-three lung cancer cases (including 204 adenocarcinoma and 212 squamous cell carcinoma) and 460 tumor-free hospital controls were investigated using PCR amplification and melting point analysis of sequence-specific hybridization probes. Odds ratios (OR) were calculated by multiple logistic regression analysis adjusting for age, gender, smoking habits, and occupational exposure and showed a slightly enhanced risk for all lung cancer cases as well as for squamous cell carcinoma and adenocarcinoma cases. Gene-environment interactions were analyzed with respect to smoking and occupational exposure. A nearly 3-fold increased risk for adenocarcinoma associated with the XPA AA genotype was observed for occupationally exposed individuals (OR, 2.95; 95% confidence interval, 1.42-6.14) and for heavy smokers (OR, 2.52; 95% confidence interval, 1.17-5.42). No genotype-dependent increase in OR was found for nonexposed individuals or those smoking <20 pack-years. The significant effect of the XPA polymorphism in heavy smokers and occupationally exposed individuals suggests an important gene-environment interaction for the XPA gene. The underlying mechanisms as to why AA homozygotes are

predisposed to lung adenocarcinoma and which specific carcinogens are involved remains to be determined. [Cancer *Epidemiology, Biomarkers & Prevention* **13** (2004) 2242-46].

Widłak W., Benedyk K., Vydra N., Głowala M., Ścieglińska D., Małusecka E., Nakai A., Krawczyk Z.: ***Expression of a constitutively active mutant of heat shock factor 1 under the control of testis-specific hst70 gene promoter in transgenic mice induces degeneration of seminiferous epithelium.***

Heat shock activates in somatic cells a set of genes encoding heat shock proteins which function as molecular chaperones. The basic mechanism by which these genes are activated is the interaction of the specific transcription factor HSF1 with a regulatory DNA sequence called heat shock element (HSE). In higher eukaryotes HSF1 is present in unstressed cells as inactive monomers which, in response to cellular stress, aggregate into transcriptionally competent homotrimers. In the present paper we showed that the expression of a transgene encoding mutated constitutively active HSF1 placed under the control of a spermatocyte-specific promoter derived from the hst70 gene severely affects spermatogenesis. We found the testes of transgenic mice to be significantly smaller than those of wild-type males and histological analysis showed massive degeneration of the seminiferous epithelium. The lumen of tubules was devoid of spermatids and spermatozoa and using the TUNEL method we demonstrated a high rate of spermatocyte apoptosis. The molecular mechanism by which constitutively active HSF1 arrests spermatogenesis is not known so far. One can assume that HSF1 can either induce or repress so far unknown target genes involved in germ cell apoptosis. [*Acta Biochim Pol.* **50** (2003): 535-541]

Widłak W., Ścieglińska D., Vydra N., Małusecka E., Krawczyk Z.: ***In vivo electroporation of the testis versus transgenic mice model in functional studies of spermatocyte-specific hst70 gene promoter: A comparative study.***

To determine whether DNA transfer to mouse testes by in vivo electroporation could be useful method for studying regulatory elements of genes specifically active in spermatocytes first we compared the expression pattern of a construct containing the EGFP reporter gene ligated to a fragment of the heat shock testis-specific hst70 gene promoter, both in testis of transgenic mice and in testis electroporated in vivo. While in transgenic mice the EGFP was expressed in all seminiferous tubules in a cell- and stage-specific manner, in the testes electroporated in vivo only small fraction of cells expressed this marker protein. In order to make a quantitative comparison between the specificity of these two experimental systems we used several vectors containing the CAT gene ligated to fragments of the hst70 gene 5' upstream of DNA sequences which either promoted or did not activate expression of the reporter gene in the testes of transgenic mice. Also, as a reference opposite to spermatogenic cells we examined the expression pattern of the same set of vectors in the rat hepatoma FTO 2B cells. Although electroporated testes retain some spermatocyte-specific features such as the ability to repress promoters which do not contain regulatory elements responsible for testis-specific transcription, several important drawbacks of the method are evident. They include basal activity of constructs which are not transcribed in testes of transgenic mice and low overall transfection efficiency. This may hamper studies in which subtle changes in the expression pattern are under investigation. However, the in vivo electroporation of the testis can be useful for preliminary screening of constructs aimed to study in transgenic mice. [*Mol Reprod Dev.* **65** (2003): 382-388]

Fiszer-Kierzkowska A., Wysocka A., Jarząb M., Lisowska K., Krawczyk Z.: ***Structure of gene flanking regions and functional analysis of sequences upstream of the rat hsp70.1 stress gene.***

We present structural and comparative analysis of the flanking regions of the rat hsp70.1 stress gene. Several repetitive sequences, microsatellites and short interspersed repetitive elements (SINEs) were found, as well as a significant gap in the 3' UTR, as compared to the orthologous mouse gene. We also show that the complex microsatellite region composed of partially overlapping inverted repeat and long homopurine-homopyrimidine sequence, which is localized 1.8 kbp upstream of the transcription start site, is capable to adopt non-B DNA structures (an H-DNA and a cruciform structure) in vitro. Functional analysis performed with the use of various fragments of the 5'end flanking regions ligated to the chloramphenicol acetyltransferase (CAT) reporter gene revealed a crucial role of cooperation between heat shock element (HSE) regulatory sequences, while none of the three HSEs alone is able to drive efficient heat induced transcription of the reporter gene. We also found that the microsatellite region does not influence transcription by itself, however, it abolishes the effect of the adjacent putative silencing element. To our knowledge, this is a first extensive structural and functional analysis of the promoter region of the mammalian heat inducible hsp70i gene localized distally to the hsp70-related spermatid-specific gene in the major histocompatibility complex III. [*Biochim Biophys Acta.* **1625** (2003): 77-87]

Menkiszak J., Gronwald J., Górski B., Jakubowska A., Huzarski T., Byrski T., Foszczynska-Kloda M., Haus O., Janiszewska H., Perkowska M., Brozek I., Grzybowska E., Zientek H., Gozdz S., Kozak-Klonowska B., Urbanski K., Miturski R., Kowalczyk J., Pluzanska A., Niepsuj S., Koc J., Szwiec M., Drosik K., Mackiewicz A., Lamperska K., Strozyk E., Godlewski D., Stawicka M., Wasko B., Bebenek M., Rozmiarek A., Rzepka-Gorska I., Narod S.A., Lubinski J.: ***Hereditary ovarian cancer in Poland***.

There is increasing evidence that hereditary factors play a greater role in ovarian cancer than in any of the other common cancers of adulthood. This is attributable, to a large extent, to a high frequency of mutations in the BRCA1 or BRCA2 genes. In Poland, 3 common founder mutations in BRCA1 account for the majority of families with identified BRCA mutations. Our study was conducted in order to estimate the prevalence of any of 3 founder BRCA1 mutations (5382insC, C61G and 4153delA) in 364 unselected women with ovarian cancer, and among 177 women with ovarian cancer and a family history of breast or ovarian cancer. A mutation was identified in 49 out of 364 unselected women with ovarian cancer (13.5%) and in 58 of 177 women with familial ovarian cancer (32.8%). The majority of women with ovarian cancer and a BRCA1 mutation have no family history of breast or ovarian cancer. The high frequency of BRCA1 mutations in Polish women with ovarian cancer supports the recommendation that all Polish women with ovarian cancer should be offered testing for genetic susceptibility, and that counseling services be made available to them and to their relatives. It is important that mutation surveys be conducted in other countries prior to the introduction of national genetic screening programs. [*Int. J. Cancer* 106 (2003): 942 - 945]

Buster D.W., Baird D.H., Yu W., Sołowska J.M., Chauviere M., Mazurek A., Kress M., Baas P.W.: ***Expression of the mitotic kinesin Kif15 in postmitotic neurons: Implications for neuronal migration and development.***

Kif15 is a kinesin-related protein whose mitotic homologues are believed to crosslink and immobilize spindle microtubules. We have obtained rodent sequences of Kif15, and have studied their expression and distribution in the developing nervous system. Kif15 is indeed expressed in actively dividing fibroblasts, but is also expressed in terminally postmitotic neurons. In mitotic cells, Kif15 localizes to spindle poles and microtubules during prometaphase to early anaphase, but then to the actin-based cleavage furrow during cytokinesis. In interphase fibroblasts, Kif15 localizes to actin bundles but not to microtubules. In cultured neurons, Kif15 localizes to microtubules but shows no apparent co-localization with actin. Localization of Kif15 to microtubules is particularly good when the microtubules are bundled, and there is a notable enrichment of Kif15 in the microtubule bundles that occupy stalled growth cones and dendrites. Studies on developing rodent brain show a pronounced enrichment of Kif15 in migratory neurons compared to other neurons. Notably, migratory neurons have a cage-like configuration of microtubules around their nucleus that is linked to the microtubule array within the leading process, such that the entire array moves in unison as the cell migrates. Since the capacity of microtubules to move independently of one another is restricted in all of these cases, we propose that Kif15 opposes the capacity of other motors to generate independent microtubule movements within key regions of developing neurons. [*J Neurocytol.* **32** (2003): 79-96]

Prosniak M., Zborek A., Scott G.S., Roy A., Phares T.W., Koprowski H., Hooper D.C.: ***Differential expression of growth factors at the cellular level in virus-infected brain.***

The contribution of host factors to rabies virus (RV) transcription/replication and axonal/transsynaptic spread is largely unknown. We previously identified several host genes that are up-regulated in the mouse brain during RV infection, including neuroleukin, which is involved in neuronal growth and survival, cell motility, and differentiation, and fibroblast growth factor homologous factor 4 (FHF4), which has been implicated in limb and nervous system development. In this study, we used real-time quantitative RT-PCR to assess the expression of mRNAs specific for neuroleukin, the two isoforms of FHF4 (FHF4-1a and -1b) encoded by the FHF4 gene, and N protein of RV in neurons and astrocytes isolated by laser capture microdissection from mouse brains infected with the laboratory-adapted RV strain CVS-N2c or with a street RV of silver-haired bat origin. Differences in the gene expression patterns suggest that the capacity of RV strains to infect nonneuronal cells and differentially modulate host gene expression may be important in virus replication and spread in the CNS. [*Proc Natl Acad Sci USA* **100** (2003): 6765-6770]

Głowala M., Mazurek A., Piddubnyak V., Fiszer-Kierzkowska A., Michalska J., Krawczyk Z.: ***HSP70 overexpression increases resistance of V79 cells to cytotoxicity of airborne pollutants, but does not protect the mitotic spindle against damage caused by airborne toxins.***

Exposure of Chinese hamster V79 cells to extracts of airborne pollutants induced formation of multipolar or incomplete mitotic spindles. To find out whether overexpression of the HSP70 chaperone protein could protect spindles against airborne toxins we constructed V79 cells stably transfected with an expression vector containing rat heat-inducible hsp70.1 gene under the control of a constitutive CMV promoter. When cells were incubated with extracts of airborne pollutants (5-20 microg/ml) no protective effect of the HSP70 protein against mitotic spindle damage was observed. Moreover, at 20 microg/ml of extracts of airborne toxins the frequency of mitotic malformations was even higher in HSP70-overexpressing cells than in control ones. Extracts of airborne pollutants of 50 microg/ml blocked the formation of mitotic figures both in control and HSP70-overexpressing cells and led to destruction of cell nuclei. However, the HSP70-overproducing cells exhibited higher survival rates when exposed to heat shock and airborne toxins than the control ones, as determined by MTT assay. This suggests that HSP70 overexpression-a frequent feature of cancer cells-should be considered as a factor facilitating survival of cells with damaged mitotic spindles and aberrantly segregated chromosomes. [*Toxicology* **170** (2002): 211-219]

Zborek A., Małusecka E., Krzyżowska-Gruca S., Wysocka A., Krawczyk Z.: ***Immunohistochemical studies on the expression pattern of molecular chaperones HSC70 and HSP25 and cell cycle-related proteins cyclin D1 and PCNA in rat liver after thioacetamide intoxication.***

Intoxication of rats with thioacetamide (TAA) is a model system to investigate mechanisms involved in liver cell death and tissue reconstitution. Our study was undertaken to determine by immunohistochemistry the expression pattern of the cytoprotective chaperone proteins HSC70 and HSP25 and proliferation markers cyclin D1 and PCNA in livers of Wistar rats intraperitoneally injected with TAA at a single dose of 50 mg/kg. For each protein studied we observed distinct dynamic changes in appearance and localization in liver lobules. During 24-36 h after TAA injection the HSC70 cytoplasmic immunoreaction gradually disappeared from hepatocytes localized around central veins and a shift of immunostaining to cell nuclei took place. Then, 36-48 h after TAA injection the HSC70 cytoplasmic immunoreaction reappeared with the highest intensity in hepatocytes surrounding the areas of inflammatory cells. HSP25, undetectable in control hepatocytes began to appear at approximately 36 h after TAA injection and HSP25-immunopositive cells formed a characteristic ring around areas of inflammation. Of the proteins studied, the most rapid reaction to TAA was observed for cyclin D1. As early as 15 min after TAA administration cyclin D1-positive hepatocytes appeared in intermediate and periportal areas of liver lobules and a subsequent shift of staining to centrilobular hepatocytes took place at 36 and 48 h. There was no correlation of cyclin D1 localization either with PCNA-positive cells or mitotic cells. Our observations suggest that in TAA-treated livers HSP25 and HSC70 proteins can play an anti-inflammatory role, and the early and distinct cyclin D1 expression is not related to proliferation of hepatocytes. [*Histochem Cell Biol.* **118** (2002): 311-319]

Grzybowska E., Sieminska M., Zientek H., Kalinowska E., Michalska J., Utracka-Hutka B., Rogozińska-Szczepka J., Kazmierczak-Maciejewska M.: ***Germline mutations in the BRCA1 gene predisposing to breast and ovarian cancers in Upper Silesia population.***

Germline mutations in the BRCA1 or BRCA2 genes predispose their carriers to breast or/and ovary cancers during their lifetime. The most frequent mutations: 5382insC, 185delAG, C61G and 4153delA in BRCA1, and 6174delT and 9631delC in BRCA2 were studied in a group of 148 probands admitted for genetic counseling, using allele-specific amplification (ASA) PCR test. Fifteen carriers of three different mutations: 5382insC, 185delAG and C61G in BRCA1 were found. Two families carried the 185delAG mutation and additional two C61G in BRCA1. Nobody carried the mutation 4153delA in BRCA1 nor 6174delT or 9631delC in BRCA2. Most of the carriers of a germline mutation were observed among the patients who developed bilateral breast cancer (17%). The lowest frequency of the germline mutations was found in the healthy persons who had two or more relatives affected with breast or ovarian cancer. [*Acta Biochim Pol.* **49** (2002): 351-356]

Forsti A., Jin Q., Grzybowska E., Soderberg M., Zientek H., Siemińska M., Rogozińska-Szczepka J., Chmielik E., Utracka-Hutka B., Hemminki K.: ***Sex hormone-binding globulin polymorphisms in familial and sporadic breast cancer.***

Ovarian steroids are one of the strongest risk factors for breast cancer. Sex hormone-binding globulin (SHBG) binds and transports sex steroids in the blood, regulating their bioavailable fraction and access to target cells. It

can also inhibit the estradiol-induced proliferation of breast cancer cells through its membrane receptor. Three coding-region polymorphisms, which lead to an amino acid change, have been reported. We studied the influence of these three polymorphisms on breast cancer risk in three different populations: Polish familial breast cancer cases, 27% of them carrying a BRCA1/BRCA2 mutation, Nordic familial and sporadic breast cancer cases. The reported G to A polymorphism in exon 1 was not found in the 423 analyzed samples. Instead, we found a C to T transition causing an arg to cys amino acid change within the same codon in one Polish breast cancer patient and her daughter. Both of them were heterozygotes for the exon 8 G to A polymorphism as well. They were diagnosed for bilateral breast cancer and carried a BRCA1 mutation (5382insC). Analysis of the tumor samples showed that they had lost the wild-type allele both at exons 1 and 8 of the SHBG gene. Analysis of the other Polish samples showed no correlation of the exon 8 polymorphism to breast cancer, bilateral breast cancer, BRCA1/BRCA2 mutations or age at diagnosis. No association of the exon 8 polymorphism with breast cancer in the Nordic familial or sporadic cases was found. The C to T polymorphism located in exon 4 was rare in all the studied populations (overall allele frequency 0.011). However, in each of the study populations there was a trend for a lower variant allele frequency in cancer cases than in controls. Variant allele frequency in all the breast cancer cases was significantly lower than in all the controls (chi(2) = 5.27, P-value 0.02; odds ratio = 0.23, 95% confidence interval 0.05-0.84). [*Carcinogenesis* **23** (2002): 1315-1320]

Sołowska J.M., Mazurek A., Weinberger L., Baird D.H.: ***Pontocerebellar axon guidance: neuropilin-1- and semaphorin 3A-sensitivity gradients across basilar pontine nuclei and semaphorin 3A variation across cerebellum.***

To assess the role of semaphorin 3A (Sema3A) and its receptor component neuropilin-1 (Npn-1) in pontocerebellar axon guidance, we compared the distributions of Sema3A, Npn-1, and DiI-labeled pontocerebellar axons in neonatal mouse cerebellum. Between embryonic day 18 and birth there was a large increase in Npn-1 expression in the basilar pontine nuclei (BPN), the major source of pontocerebellar axons. Sema3A expression in cerebellum also increased at this time. In the BPN, Npn-1 and the response of axons to Sema3A were graded with high Npn-1 and Sema3A responsiveness rostrally and lower levels caudally. The Npn-1 gradient was not smooth and cells with higher and lower expression were interspersed. Between birth and postnatal day 5, pontocerebellar axons projected to lobules of the hemispheres, including those with low to moderate levels of Sema3A, but did not enter regions with high levels of Sema3A, including the flocculus and much of the vermis. These results suggest that varying neuropilin levels on BPN axons, which correlated with their varying responses to Sema3A, combined with varying Sema3A levels across cerebellum, may contribute to guiding subsets of BPN axons to their distinct target regions within cerebellum. [*Mol Cell Neurosci.* **21**(2002): 266-284]

Spitsin S.V., Scott G.S., Mikheeva T., Zborek A., Kean R.B., Brimer C.M., Koprowski H., Hooper D.C.: ***Comparison of uric acid and ascorbic acid in protection against EAE***.

Serum levels of uric acid (UA), an inhibitor of peroxynitrite- (ONOO-) related chemical reactions, became elevated approximately 30 million years ago in hominid evolution. During a similar time frame, higher mammals lost the ability to synthesize another important radical scavenger, ascorbic acid (AA), leading to the suggestion that UA may have replaced AA as an antioxidant. However, in vivo treatment with AA does not protect against the development of experimental allergic encephalomyelitis (EAE), a disease that has been associated with the activity of ONOO- and is inhibited by UA. When compared in vitro, UA and AA were found to have similar capacities to inhibit the nitrating properties of ONOO-. However UA and AA had different capacities to prevent ONOO- -mediated oxidation, especially in the presence of iron ion (Fe3+). While UA at physiological concentrations effectively blocked dihydrorhodamine-123 oxidation in the presence of Fe3+, AA did not, regardless of whether the source of ONOO- was synthetic ONOO-, SIN-1, or RAW 264.7 cells. AA also potentiated lipid peroxidation in vivo and in vitro. In conclusion, the superior protective properties of UA in EAE may be related to its ability to neutralize the oxidative properties of ONOO- in the presence of free iron ions. [*Free Radic Biol Med.* **33** (2002): 1363-1371]

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Current research interest:
The research projects conducted in the Department are focused on molecular mechanisms involved in various thyroid disorders: hereditary and somatic mutations, polymorphisms and changes in gene expression and implementation of molecular data into clinical practice. The second research area is associated with novel approaches in the treatment of thyroid cancer.
The main topics are:

- Gene expression profiling in tumors;
- Genetic predisposition to medullary thyroid carcinoma and pheochromocytoma, clinical consequences of molecular data;
- Somatic mutations and chromosomal rearrangements in papillary and medullary thyroid carcinomas;
- Estimation of the thyroglobulin role as an early marker of differentiated thyroid carcinoma: a study of thyroglobulin concentration in the thyroid and serum as well as *TG* RT-PCR estimation for detection of lymph node metastases;
- Association of polymorphisms in LTalpha, TNF, CTLA4, IL-4 and IL-10 genes in Graves' and/or Hashimoto diseases;
- The novel approaches in the treatment of differentiated thyroid cancer and its metastases: the use of 13-cis retinoic acid, the use of recombinant human TSH, intraoperative isotope detection of thyroid carcinoma, evaluation of effects of L-thyroxine therapy;
- Radioimmunotherapy with anti-EGFR antibody labeled with ^{125}I.

Selected Papers:

Wiench M, Wloch J, Wygoda Z, Gubala E, Oczko M, Pawlaczek A, Kula D, Lange D, Jarząb B. ***RET polymorphisms in codons 769 and 836 are not associated with predisposition to medullary thyroid carcinoma.***

The study was undertaken to verify whether the RET gene polymorphisms are associated with MTC in patients negative for germline mutations. Two hundred five patients with apparent sporadic MTC were subjected to genetic analysis of RET exons 10, 11, 13, 14, 16 and 22 RET germline mutation carriers were identified with 10.7% frequency. The frequency among 26 patients not older than 30 was 27%. In patients excluded for known mutations we analyzed two polymorphic sites: RET codon 769 and 836. As control group, 90 healthy subjects were investigated. In young patients the observed allelic frequencies were 32% for variant L769/CTG and 5% for variant S836/AGT. Although these values were higher than in older MTC patients (22 and 3%, respectively), as well as in the control group (27 and 2%) the difference was insignificant. We conclude that in Polish patients polymorphisms at RET codons 769 and 836 are not associated with medullary thyroid carcinoma. [*Cancer Detect Prev*. 28 (2004): 231-6].

Schlumberger M, Pacini F, Wiersinga WM, Toft A, Smit JW, Sanchez Franco F, Lind P, Limbert E, Jarząb B, Jamar F, Duntas L, Cohen O, Berg G. ***Follow-up and management of differentiated thyroid carcinoma: a European perspective in clinical practice.***

As differentiated (follicular and papillary) thyroid cancer (DTC) may recur years after initial treatment, follow-up of patients with DTC is long term. However, this population has changed, with more individuals being discovered at an earlier stage of disease, so that previous follow-up protocols based mostly on data from high-risk patients no longer apply. We have proposed, in a previous issue of this Journal, an improved protocol for the follow-up of low-risk patients with DTC based on the findings of recent studies. We report here the case of a paradigmatic patient with papillary thyroid carcinoma, with the goal of illustrating the benefits of applying this algorithm in routine clinical practice. We also offer expanded and additional comments on various issues in the management of DTC. [*Eur J Endocrinol***. 151** (2004): 539-48].

Hendryk S, Jarząb B, Josko J. ***Increase of the IL-1 beta and IL-6 levels in CSF in patients with vasospasm following aneurysmal SAH.***

Cytokines play a key role in mutual influence of the immunological, endocrine and CNS systems. It has been proven that proinflammatory ILs may intensify the cascade of biochemical changes in ischemic brain damage. Vasospasm, which may accompany SAH and often coexists with symptoms of DINDs, is the cause of ischemic changes in the brain. It is thought that immunological mechanisms may be one of the causes of degenerative-productive changes in vessel walls, in delayed vasospasm following SAH, which lead to substantial vasospasm and in consequence too cerebral ischemia. In the randomly selected group of patients, who underwent surgical treatment after aneurysmal SAH, we determined the concentration of IL-1 beta and IL-6 in CSF in the periods between Days 0 to 3; 4 to 7; and 8 to 15 after the occurrence of SAH. The presence and dynamics of development of vasospasm were assessed on the basis of increasing DINDs as well as CT and cerebral angiography. We examined the concentrations of ILs in CSF using radioimmunological methods, applying commercially available tests for their assessment. We found that in the period between 8 and 15 days after SAH, in increasing delayed vasospasm and DINDs, here is a statistically significant increase concentration of IL-1 beta in CSF (105.4 +/- 46.9 pg x ml-1; $p<0.005$), and no significant changes in patients without vasospasm and neurological deficits. On the other hand, we noted a statistically significant increase concentration of IL-6 in CSF (4802 +/- 1170 ng x ml-1; $p<0.05$) only in the acute phase after SAH (Days 0-3) in patients in poor clinical condition, in whom delayed vasospasm and cerebral ischemia developed later. This increase of ILs level in CSF is probably related to the intensity of the SAH, and secondarily aggravates the vasospasm and ischemic changes in the brain. [*Neuro Endocrinol Lett.* **25** (2004): 141-7].

Schlumberger M, Berg G, Cohen O, Duntas L, Jamar F, Jarząb B, Limbert E, Lind P, Pacini F, Reiners C, Sanchez Franco F, Toft A, Wiersinga WM. ***Follow-up of low-risk patients with differentiated thyroid carcinoma: a European perspective.***

OBJECTIVE: Because differentiated (follicular and papillary) thyroid cancer (DTC) may recur years after initial treatment, the follow-up of patients with DTC is long term. However, this population has changed, with more individuals being discovered at an earlier stage of the disease, so that previous follow-up protocols based mostly on data from high-risk patients no longer apply. We sought to develop an improved protocol for the follow-up of low-risk patients with DTC based on the findings of recent studies. METHODS: We analysed recent literature on

the follow-up of DTC. RESULTS: Recent large studies have produced three important findings: (i) in patients with low-risk DTC with no evidence of disease up to the 6- to 12-month follow-up, diagnostic whole-body scan adds no information when serum thyroglobulin (Tg) is undetectable and interference from anti-Tg antibodies is absent; (ii) use of recombinant human thyroid-stimulating hormone to aid Tg measurement is effective and provides greater safety, quality-of-life and work productivity than does levothyroxine withdrawal with its attendant hypothyroidism; and (iii) ultrasonography performed by an experienced operator is the most sensitive means of detecting neck recurrences of DTC. CONCLUSIONS: We present a revised follow-up protocol for low-risk patients taking into account the above findings. This protocol should help clinicians enter a new era of monitoring characterized by greater safety, simplicity, convenience and cost savings. [*Eur J Endocrinol*. **150** (2004): 105-12].

Jarząb B., Handkiewicz-Junak D., Roskosz J., Puch Z., Wygoda Z., Kukulska A., Jurecka-Lubieniecka B., Hasse-Lazar K., Turska M., Zajusz A.: ***Recombinant human TSH-aided radioiodine treatment of advanced differentiated thyroid carcinoma: a single-centre study of 54 patients.***

We sought to evaluate the efficacy, biochemical effects, safety and outcome of recombinant human thyroid-stimulating hormone (rhTSH) as an adjunct to radioiodine treatment of advanced differentiated thyroid carcinoma (DTC). We also sought to determine whether rhTSH is useful as an adjunct to radioiodine treatment following isotretinoin re-differentiation therapy of DTC metastases that have lost function. Therefore, in 54 consecutive patients who had retained bulky metastatic and/or locoregional lesions of DTC despite the exhaustion of other therapeutic options, we gave one to four courses of two consecutive daily intramuscular injections of rhTSH, 0.9 mg, followed by a therapeutic activity of (131)I per os on day 3. Fifty patients had received prior radioiodine treatment aided by l-thyroxine (T(4)) withdrawal. We included in the study 23 patients who had received a trial of isotretinoin therapy for re-differentiation of confirmed de-differentiated metastases. In a blinded, within-patient comparison of post-therapy whole-body scans after the first rhTSH-aided and latest withdrawal-aided treatments in patients with functional metastases at baseline, 18 of 27 (67%) scan pairs were concordant, four (15%) were discordant in favour of the rhTSH-aided scan and five (19%) were discordant in favour of the withdrawal-aided scan. In total, 37 (74%) of 50 paired scans were concordant, eight (16%) favoured rhTSH and five (10%) favoured withdrawal. All differences appeared to be attributable to clinical causes, not to any difference between endogenous and exogenous TSH stimulation. Reflecting the biochemical activity of rhTSH and the release of thyroglobulin (Tg) due to tumour destruction, median serum Tg concentration rose approximately fourfold between baseline and day 6 of the rhTSH-aided treatment course. rhTSH was well tolerated, with mostly minor, transient toxicity, except for neck oedema in three patients with neck infiltrates and pathological spine fracture in one patient with a large vertebral metastasis. At 6 months, complete response occurred in one (2%), partial response in 12 (26%) and disease stabilisation in 19 (40%) of 47 evaluable patients. The rate of complete + partial response was 41% and that of disease stabilisation, 30%, in the 27 evaluable patients with functional metastases at baseline; the corresponding rates were 10% and 55% in the 20 evaluable patients with non-functional metastases at baseline. Although within-patient comparison of early outcome after both modalities is limited by a significantly greater median number of courses and a greater median cumulative activity of radioiodine given under withdrawal, response to rhTSH-aided and withdrawal-aided treatment was similar in 23 (52%) of 44 evaluable patients, superior with rhTSH in 12 (27%) and superior with withdrawal in seven (16%). In two patients, a superior response was obtained after isotretinoin pretreatment and rhTSH and attributed to re-differentiation therapy. In conclusion, our study provides preliminary evidence that rhTSH safely and effectively aids radioiodine treatment of advanced DTC, and does so to an at least equivalent degree as does T(4) withdrawal. [*Eur. J. Nucl. Med. Mol. Imaging* **30** (2003) 1077-1086]

Wygoda Z., Tarnawski R., Brady L., Stęplewski Z., Bazowski P., Wojtacha M., Stepień T., Kula D., Składowski K., Kokocinska D., Wygoda A., Pawlaczek A., Etmanska A., Larysz D., Jarząb B.: ***Simultaneous radiotherapy and radioimmunotherapy of malignant gliomas with anti-EGFR antibody labelled with iodine 125. Preliminary results.***

BACKGROUND: In this paper we present the preliminary results of a prospective trial of the efficacy of simultaneous radiotherapy and anti-EGFR (125)I radioimmunotherapy of malignant gliomas with 2 years' total survival as the end-point, raising the question whether anti-EGFR (125)I radioimmunotherapy influences the disease-free survival in these patients. MATERIAL AND METHODS: Patients with anaplastic astrocytoma or primary glioblastoma were previously treated by a macroscopically radical neurosurgical approach and randomized either to radiotherapy + radioimmunotherapy arm or treated by radiotherapy alone. Seven patients were included in the group with radioimmunotherapy, among them five with GBM and two with AA, and five patients in the control arm. Patients were irradiated to 60 Gy using three-dimensional conformal noncoplanar

techniques. Anti-EGFR (125)I monoclonal antibody 425 radioimmunotherapy (50mCi/course) was started during 4th week of radiotherapy and was repeated three times in one week intervals. RESULTS: Time of follow-up ranges between 2 and 10 months in the anti-EGFR (125)I radioimmunotherapy arm and 4 and 9 months in the control arm. Recurrence was diagnosed in all patients in the EGFR (125)I group with a lethal outcome in two of them and in 4 patients in the control group. Median time to recurrence was 2 and 5 months respectively. CONCLUSIONS: Taking into account early recurrences observed, we propose to continue the studies on the efficacy of adjuvant anti-EGFR (125)I radioimmunotherapy in a selected group of patients in whom the greatest benefit may be expected on the basis of molecular studies, among them EGFR expression investigation. [*Nucl. Med. Rev. Cent. East Eur*. **5** (2002) 29-33]

Hendryk S., Jędrzejowska-Szypulka H., Josko J., Jarząb B., Dohler K.D.: ***Influence of the corticotropin releasing hormone (CRH) on the brain-blood barrier permeability in cerebral ischemia in rats.***

The increase in the blood-brain barrier (BBB) permeability and a developing cerebral oedema due to the ischemic infarction appear a few hours, and intensify during a few days, after closing the carotid arteries. It fails to be clear, however, what causes the increase in the microvessels damage, and whether the damage is a secondary result of the vasoactive substances released by the neurones and glia cells damaged by the ischemia. CRH, which plays an essential role in integrative the nervous, endocrine, and immunological systems, has a positive effect on the decrease in the permeability of the BBB damaged by various physical and chemical factors. Therefore, the examination of the CRH role in the cerebral ischemia may prove useful for explaining the processes taking place in the foci of the cerebral infarction and their environment. The experiment was carried out on rats which, 20 minutes before closing of both internal carotid arteries, was administered 10 microg CRH to cerebrospinal fluid via cisterna magna of the brain. The BBB permeability was measured 30 minutes, 3 hours, 3 days, and 7 days after closing the arteries. The experiment has shown the CRH protective effect on the BBB and its consequent effect on the decrease in the BBB permeability which appears in the 3 hours after closing the arteries ($p<0.05$), and is high significant during the chronic phase of the cerebral ischemia ($p<0.03$). It can be thus concluded that CRH, by affecting directly the endothelium of the cerebral vessels, decreases the endothelial damage in the acute phase of the ischemia. The decrease is noted to be more significant in the chronic phase of the ischemia; such an effect can be attributed to CRH stimulating the hypothalamic-adrenal axis, and to the secondary activation of the mechanisms decreasing the BBB permeability. [*J. Physiol. Pharmacol*. **53** (2002) 85-94]

Czarnywojtek A., Krysinska I., Lacka K., Stawny B., Rolski M., Jarząb B., Włoch J., Gembicki M.: ***A study of thyroglobulin concentration in the thyroid and serum of patients with different thyroid disorders***

Knowledge concerning the structure and quality of thyroglobulin (Tg) has great significance for the better understanding of the pathogenesis of different thyroid diseases. The localization of the Tg gene and studies of its structure by molecular biological techniques make possible precise investigations of its expression. The aim of our study was to evaluate Tg content in the thyroids and Tg concentrations in the serum of 108 patients suffering from benign or malignant thyroid disorders. The method of investigation was isolating total protein from thyroid tissues obtained during surgery and determining Tg content in the thyroid extracts and Tg concentrations in serum. The Tg concentrations in serum and in thyroid protein extracts were evaluated by fluoroimmunometric assay. Statistical analysis was carried out with the help of the computing programmes. [*Arch. Immunol. Ther. Exp*. **50**; (2002) 143-148]