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Division of Experimental Oncology

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

Scientific Report 2001-2003

Department of Cancer Epidemiology and Regional Silesian Cancer Registry

The Department conduct the following activities:

- Monitoring of the cancer cases within Upper Silesia District (the monitoring of cancer ills based on obligatory procedure on reporting them to the Regional Silesian Cancer Registry, on a form MzN1a – identical for the whole Poland). Registry is fully computerised.
- Descriptive epidemiology of cancers: incidence and mortality by age, gender; age-specific rates, crude and age-adjusted rates; oncocartography for small areas – community councils, counties – for a selected and the most frequent cancer sites: lung cancer (males, females), breast cancer in females, genitourinary organs in female and male population of Silesia; a detailed geography of cancers.
- Analytical epidemiology of cancers:
  Based on the Mantel-Haenszel procedure – relative risk rate; identification of an important risk factor which are the cause of lung cancers, cervix and corpus uteri, breast, skin and larynx cancers.

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Selected Papers:

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]

Wojcieszek E., Wojarska-Tręda E., Kołosza Z., Woźniakowska W., Felcenloben M., Laskowska M., Basek E.: ***Haemodynamic effects of fentanyl and remifentanil during anaesthesia for oncology surgery***.

The purpose of the study was to assess the usefulness of remifentanil in oncology surgery, and its effects on hemodynamics at various stages of general anaesthesia. Sixty-seven adult ASA I and II patients, of both sexes, were randomly allocated to two groups to receive either fentanyl or remifentanil during propofol-N_2O anaesthesia. The fentanyl group received a 5 mcg kg^{-1} bolus dose followed by 100-200 mcg increments while the remifentanil group received 0.4 mcg kg^{-1} min^{-1} initial dose , followed by 0.2 mcg kg^{-1} min^{-1} infusion. Both groups received either atracurium or vecuronium for muscle relaxation. The non-parametric Mann-Whitney test was used for statistical analysis. ABP measured after skin incision was significantly lower ($p=0.018$) in the remifentanil group. Heart rate was also lower in this group. Patients of the remifentanil group who received more than 250 mg of propofol during anaesthesia, had a significantly lower ABP during recovery than those who received a lower dose. During recovery ABP in the fentanyl group was lower than in the remifentanil group, possibly indicating inadequate analgesia in this period. The use of remifentanil is associated with a less pronounced cardiovascular reaction on intubation and skin incision. In patients recovering from remifentanil-based anaesthesia, postoperative analgesia should be started early. [*Anestezjologia Intensywna Terapia* **36** (2003) 88-95*, in Polish*]

Juszko-Piekut M., Kołosza Z., Moździerz A.: ***Alcohol and the diet as risk factors of pulmonary carcinoma among original inhabitans of the former Katowice Province.***

Estimation of the risk of pulmonary occurrence among men was done by case-control study of autochthons men population considering consumption of alcohol and selected elements of the diet. Value of the relative risk was calculated by Mantel-Haenszel test. As a result it was found that consumption of such products as fish, vegetables, pickles, butter, margarine, boiled meat as well as lack fried meat in the diet decrease risk of pulmonary carcinoma occurrence caused by strong alcohol consumption. Influence of white bread and potatoes consumption on the risk of the carcinoma occurrence was not found. [*Polish Journal of Human Nutrition and Metabolism,* **XXX** (2003) 800-804*, in Polish*]

Juszko-Piekut M., Kołosza Z., Moździerz A.: Elements of the diet as factors decreasing risk of pulmonary carcinoma.

Case-control study was performed including examination of 461 men suffering from pulmonary carcinoma and 660 not suffering from any cancer, living in the industrial area (former Katowice Province). It was done to estimate dependence between pulmonary carcinoma and selected elements of the diet. Value of the relative risk (odds ratio) and 95% confidence interval for selected elements of the diet were calculated by logistic regression method. Men whose diet contained raw vegetables, oil, butter, margarine, boiled meat, fish and pickles revealed lower risk of pulmonary carcinoma than men whose diet lacked in m/a elements (didn't eat or eat very seldom).

Such dependencies were not found in case of white bread and potatoes. [*Polish Journal of Human Nutrition and Metabolism,* **XXX** (2003) 805-809*, in Polish*]

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]

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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Selected Papers:

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]

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*. (2003): in press]

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]

Horak S., Polańska J., Widłak P.: ***High levels of bulky DNA adducts in human sperm correlate with impaired fertility.***
Progressive decline in fertility and sperm quality has been reported over the last few decades, especially in industrialized nations. It has been proposed that exposure to factors that induce damage in DNA of spermatogenic cells may significantly contribute to impaired fertility. Here, the 32P-postlabelling method was used to analyze the levels of bulky DNA adducts in sperm cells in a group of 179 volunteers, either healthy subjects or patients with an impaired fertility. The levels of DNA adducts were 1.35-fold higher in the infertile group as compared to healthy individuals ($P = 0.012$). Similarly, a significant negative correlation between the levels of DNA adducts and measures of semen quality (sperm concentration and motility) has been observed (P=0.001). In addition, the levels of bulky DNA adducts in sperm cells positively correlates with amounts of leukocytes in semen, which were significantly higher in semen of infertile subjects. [*Acta Biochim. Polon*. **50** (2003) 197-203]

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]

Konopacka M., Rzeszowska-Wolny J.: ***Antioxidant Vitamins C, E and β-carotene reduce DNA damage before as well as after γ-ray irradiation of human lymphocytes in vitro.***

The protective effect of Vitamins C, E and beta-carotene against gamma-ray-induced DNA damage in human lymphocytes in vitro was investigated. Cultured lymphocytes were exposed to increasing concentration of these vitamins either before or after irradiation with 2Gy of gamma-rays and DNA damage was estimated using micronucleus assay. A radioprotective effect was observed when antioxidant vitamins were added to cultured cells before as well after irradiation; the strongest effect was observed when they were added no later than 1h after irradiation. The radioprotective effect of vitamins also depended on their concentration; Vitamins C added at low concentration (1 microg/ml) before exposure of the cells to radiation prevented induction of micronuclei. Vitamin E at the concentration above 2 microg/ml decreased the level of radiation-induced micronuclei when compared to the cells irradiated without vitamin treatment. beta-Carotene was effective at all tested concentrations from 1 to 5 microg/ml and reduced the number of micronuclei in irradiated cells. The vitamins had no effect on radiation-induced cytotoxicity as measured by nuclear division index. The radioprotective action of antioxidant Vitamins C, E and beta-carotene was dependent upon their concentration as well as time and sequence of application. [*Mutat. Res.* **491** (2001) 1-7]

Wideł M., Kołosza Z., Jędruś S., Łukaszczyk B., Raczek-Zwierzycka K., Świerniak A.: ***Micronucleus assay in vitro provides significant prognostic information in human cervical carcinoma; the updated analysis.***

Purpose: Reanalysis after a 5 – year follow-up previously presented relationship between spontaneous and radiation – induced micronucleus frequencies in tumour cells and the clinical outcome of patients with advanced stages (II B-IVB) of cervix carcinomas treated with radiotherapy. Materials of methods: Spontaneous and induced in vitro and in vivo micronucleus frequencies were determined and related to clinical parameters . Data were analysed by the univariate Kaplan-Meier method and multivariate Cox proportional hazards model. Results: In univariate analysis stage, spontaneous micronucleus frequency before radiotherapy (MNSP) and per cent increment of micronucleus level in vivo after 20 Gy in relation to spontaneous pretreatment level were statisically significant predictors of 5 – year recurrence-free, disease – free and overall survival. Neither micronucleus frequency (MN/BNC at 2 Gy) nor proliferating fraction (%BNC at 0 Gy) estimated in vitro (in primary culture) were related to radiotherapy outcome. The age of patients was not associated with clinical results. Multivariate analysis demonstrated that the clinical stage of disease, the high frequency of spontaneous micronuclei and low-inducedmicronucleus frequency were independent and significantly unfavourable predictive factors for disease-free and overall survival. But for local control, only high MNSP and low-induced MN frequency were significant negative predictive variables. Conclusions: A high frequency of micronuclei before radiotherapy and a slight increase of micronucleus frequency during raditherapy measured after 10 fractions of 2 Gy were independent on stage, statistically significant adverse pradictors of clinical outcome in cervival carcinoma patients treated with radiotherapy. [*Int. J. Radiat. Biol*. **77** (2001) 631-635]

Widłak P., Li L.Y., Wang X., Garrard W.T.: ***Action of recombinant human apoptotic endonuclease G on naked DNA and chromatin substrates; cooperation with exonuclease and DNaseI.***

Endonuclease G (endoG) is released from mitochondria during apoptosis and is in part responsible for internucleosomal DNA cleavage. Here we report the action of the purified human recombinant form of this endonuclease on naked DNA and chromatin substrates. The addition of the protein to isolated nuclei from nonapoptotic cells first induces higher order chromatin cleavage into DNA fragments > 50 kb in length, followed by inter- and intranucleosomal DNA cleavages with products possessing significant internal single-stranded nicks spaced at nucleosomal (~190 bases) and subnucleosomal (~10 bases) periodicities. We demonstrate that both exonucleases and DNase I stimulate the ability of endoG to generate double-stranded DNA cleavage products at physiological ionic strengths, suggesting that these activities work in concert with endoG in apoptotic cells to ensure efficient DNA breakdown. [*J. Biol. Chem*., **276** (2001) 48404–48409]

Widłak P., Garrard W.T.: ***Ionic and cofactor requirements for the activity of the apoptotic endonuclease DFF40/CAD.***

The endonuclease DFF40/CAD mediates regulated DNA fragmentation and chromatin condensation in cells undergoing apoptosis. Here we report the enzyme's co-factor requirements, and demonstrate that the ionic changes that occur in apoptotic cells maximize DFF40/CAD activity. The nuclease requires Mg2+, exhibits a trace of activity in the presence of Mn2+, is not co-stimulated by Ca2+, is inhibited by Zn2+ or Cu2+, and has high activity over a rather broad pH range (7.0-8.5). The enzyme is thermally unstable, and is rapidly inactivated at 42 degrees C. Enzyme activity is markedly affected by ionic strength. At the optimal [K+] of 50-125 mM, which is in the range of the cytoplasmic [K+] for cells undergoing apoptosis, the activity of DFF40/CAD for naked DNA cleavage is about 100-fold higher than at 0 or 200 mM [K+]. Although these ranges of ionic strength do not affect DFF40 homo-oligomer formation, at higher ionic strengths the enzyme introduces single-stranded nicks into supercoiled DNA. [*Mol. Cell. Biochem*. **218 (2001)** 125-130]

Świerniak A., Kimmel M., Śmieja J., Rzeszowska-Wolny J.: ***Modelling of cell aging system - theoretic approach.***

We characterize the asymptotic behavior of telomeres shortening of which is supposed to be the mechanism of aging and death. The problem is described by models in the form of infinitely many differential linear first order equations, resulting from branching random walk processes used to represent the evolution of particles in this problem, under different assumptions dealing witch stochastic characterization of the process. We use control theoretical machinery based on Laplace transforms, Tauberian theorems and transfer loop reduction. [*J. Med. Inf. Technol*. **2** (2001) MI3-8]

Fujarewicz K., Kimmel M., Rzeszowska-Wolny J., Świerniak A.: ***Improved classification of microarray gene expression data using support vector machine.***

Microarrays are new technicque of gene expression measurements that attracted a great deal of research interest in recent years, It has been suggested that gene expression data from microarrays (biochips) can be utilized in many biomedical areas, for example in cancer classification. Whereas several, new and existing, methods of classification has been tested, a selection of proper (optimal) set of genes, which expression serves during classification, is still an open problem. In this paper we propose a heuristic method of choosing suboptimal set of genes by using support vector machines (SVMs). Obtained set of genes optimizes one-leave –out cross-validation error. The method is tested on microarray gene expression data of samples of two cancer types: acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). The results show that quality of classification of selected set of genes is much better than for sets obtained using another methods of feature selection. [*J. Med. Inform. Technol*. **2** (2001) MI9-17]

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 genes and proteins in combination with chemo- and radiotherapy modalities.

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

1. antiangiogenic gene therapy based on genes encoding several antiangiogenic proteins (angiostatin, endostatin, vasostatin, etc.) in combination with chemo- or radiotherapy for the treatment of primary and metastatic tumors

2. Construction of novel drug conjugates as well as liposomal and polymeric drug carriers that contain RGD ligands enabling recognition of $\alpha_V\beta_3$ integrins.

3. Recombinant antiangiogenic proteins isolated from *E. coli* or yeasts

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Selected Papers:

Sochanik A., Cichon T., Makselon M., Stróżyk M., Smolarczyk R., Jazowiecka J. Szala S.: ***Cetylated polyethylenimine in gene transfer in vivo***.
This report describes gene transfer in vivo accomplished using cetylated low-molecular weight (600Da) polyethylenimine (28% of amine groups substituted with cetyl moieties). Due to its hydrophobic nature this compound has be used in the form of liposomes in order to be suitable for gene transfer studies. Serum-induced plasmid DNA degradation assay demonstrates that DNA is well protected (through condensation) by CT-PEI-containing liposomal carriers. Comparison of in vitro luciferase gene expression in cells grown in medium

supplemented with 10% serum and in medium that is serum-reduced showed that they were comparable. Expression was highest using CT-PEI/cholesterol liposomes followed by CT-PEI/DOPE liposomes and by (uncetylated) PEI 600Da carrier. We thus attempted next an in vivo transfer of complexes containing CT-PEI liposomes. Mice were administered intravenously complexes containing liposome formulations made of CT-PEI and either dioleoylphosphatidylcholine/cholesterol or cholesterol alone. Use of the latter formulation resulted in higher luciferase expression (and more so in lungs than in liver). In conclusion: liposomes containing cetylated polyethylenimine and cholesterol are a suitable vehicle for systemic plasmid DNA transfer into lungs. [*Acta Biochim. Polon.* **50** (2003) in press]

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]

Szary J., Kalita K., Przybyszewska M., Duś D., Kieda C., Janik P. Szala S.: ***KDR Promoter can transcriptionally Target Cytosine deaminase Suicide gene to Cancer cells of Nonendothelial Origin.***

The KDR/flk-1 gene promoter is considered to be endothelial cell-specific. We show its activity in two cancer cell lines of non-endothelial origin: in murine L1 sarcoma and OVP-10 human ovarian carcinoma cell lines. KDR promoter driven cytosine deaminase gene can be efficiently expressed in these cells leading to sensitization to 5-fluorocytosine, as demonstrated both *in vitro* and *in vivo*. Our results indicate that KDR promoter activity is not endothelial cell-exclusive and that this promoter can also be used to obtain specific expression of therapeutic genes in certain cancer cells. [*Anticancer Research* **21** (2001) 3471-3476]

Szary J. Szala S.: ***Intra-tumoral administration of naked plasmid DNA encoding mouse endostatin inhibits renal carcinoma growth.***

Endostatin is a C-terminal fragment of collagen XVIII and has potent anti-angiogenic and anti-tumor activity. Mouse endostatin-coding sequences were obtained using PCR and linked to the signal sequence of influenza virus hemagglutinin. The signal-sequence endostatin fragment was subcloned into plasmid vectors under the transcriptional control of cytomegalovirus promoter. Muroine renal carcinoma (Renca) cells transfected with endostatin-coding plasmid are shown to secrete full-length endostatin. Endostatin-secreting Renca cells have demonstrated slower growth *in vivo* compared to empty vector-transfected cells. but their *in vitro* growth is unaffected. Antiangiogenic activity of secreted endostatin was confirmed in a Matrigel angiogenesis assay *in vivo*. We report growth inhibition of Renca tumors resulting from intra-tumoral delivery of plasmid vector encoding secretable endostatin. Elevated local concentrations of endostatin resulted from multiple intra-tumoral injections of endotoxin-purified plasmid DNA. Local endostatin levels were high enough to obtain growth arrest of Renca tumors. [*Int. J. Cancer* **91** (2001) 835-839].

Department of Tumor Biology

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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:

Seker H., Butkiewicz D., Bowman E.D., Rusin M., Hedayati M., Grossman L., Harris C.C.: ***Functional significance of XPD polymorphic variants: attenuated apoptosis in human lymphoblastoid cells with the XPD 312 Asp/Asp genotype.***

Recent molecular epidemiological studies have identified polymorphisms in the XPD gene that are associated with increased risk of brain gliomas and head, neck, lung, and skin cancers. However, the functional significance of these polymorphic variants in altering cell processes such as cell cycle checkpoints, DNA repair, and apoptosis is uncertain. We have cloned the XPD variants Lys751Gln, Asp312Asn, and Lys751Gln-Asp312Asn into a pcDNA-3.1-expression vector. Using these constructs, we did not find any detectable difference in either in vitro binding with wild-type p53 or in DNA repair proficiency as measured by host cell reactivation assay. We then genotyped 34 different lymphoblastoid cell lines from six Centre d'Etude du Polymorphisme Humaine (CEPH)/Utah pedigree families and a CEPH/French pedigree family for polymorphisms at codons 751 and 312 and assessed their apoptotic response after either UV or ionized radiation exposure. The lymphoblastoid cell lines with homozygous or heterozygous Asp at codon 312 have similar apoptotic rates, whereas cell lines with homozygous Asn at codon 312 showed a 2.5-fold increased response to UV ($P = 0.005$; Student's t test). This is the first report known to us of a functional polymorphism in a gene involved in DNA damage-induced apoptosis. However, the presence of Lys or Gln at codon 751 did not influence the apoptotic response to UV. The diminished apoptotic response of cells containing the 312 Asp allele could both allow the survival and selective clonal expansion of carcinogen-damaged cells and be a mechanistic explanation for the increased risk of cancer at diverse tissue sites. [*Cancer Res.* **61** (2001): 7430-7434]

Motykiewicz G., Małusecka E., Michalska J., Kalinowska E., Włoch J, Butkiewicz D., Mazurek A., Lange D., Perera F.P., Santella R.M.: ***Immunoperoxidase detection of polycyclic aromatic hydrocarbon-DNA adducts in breast tissue sections.***

Although the etiology of the majority of human breast cancers is unknown, environmental carcinogens are suspected to play a role. In this study, we investigated polycyclic aromatic hydrocarbon-DNA adducts in 78 breast cancer patients and benign breast disease patients with lifetime environmental exposure to polycyclic aromatic hydrocarbon (PAH) compounds. Adducts were detected in paraffin sections by immunoperoxidase method using polyclonal antiserum and were quantitated by the image-analyzing system. A significantly higher level of adducts was found in benign breast disease as compared to cancer patients ($P < .001$; Mann-Whitney U test). Neither smoking nor genetic polymorphisms in glutathione S-transferase and cytochrome P450 influenced

the level of adducts. This exploratory study demonstrates the usefulness of the immunoperoxidase method to detect PAH-DNA adducts in stored breast tissue and suggests further research on a larger population, including patients from both high- and low-pollution environments. [*Cancer Detect Prev.* **25** (2001): 328-335]

Butkiewicz D., Rusin M., Enewold L., Shields P.G., Chorąży M., Harris C.C.: ***Genetic polymorphisms in DNA repair genes and risk of lung cancer.***
Polymorphisms in DNA repair genes may be associated with differences in the repair efficiency of DNA damage and may influence an individual's risk of lung cancer. The frequencies of several amino acid substitutions in XRCC1 (Arg194Trp, Arg280His and Arg399Gln), XRCC3 (Thr241Met), XPD (Ile199Met, His201Tyr, Asp312Asn and Lys751Gln) and XPF (Pro379Ser) genes were studied in 96 non-small-cell lung cancer (NSCLC) cases and in 96 healthy controls matched for age, gender and cigarette smoking. The XPD codon 312 Asp/Asp genotype was found to have almost twice the risk of lung cancer when the Asp/Asn + Asn/Asn combined genotype served as reference [odds ratio (OR) 1.86, 95% confidence interval (CI), 1.02-3.40]. In light cigarette smokers (less than the median of 34.5 pack-years), the XPD codon 312 Asp/Asp genotype was more frequent among cases than in controls and was associated with an increased risk of NSCLC. Compared with the Asn/Asn carriers, the OR in light smokers with the Asp/Asn genotype was 1.70 (CI0.35 0.43-6.74) and the OR in those with the Asp/Asp genotype was 5.32 (CI0.35-21.02) (P trend = 0.01). The 312 Asp/Asp genotype was not associated with lung cancer risk in never-smokers or heavy smokers (>34.5 pack-years). The XPD-312Asp and -751Lys polymorphisms were in linkage disequilibrium in the group studied; this finding was further supported by pedigree analysis of four families from Utah. The XPD 312Asp amino acid is evolutionarily conserved and is located in the seven-motif helicase domain of the RecQ family of DNA helicases. Our results indicate that these polymorphisms in the XPD gene should be investigated further for the possible attenuation of DNA repair and apoptotic functions and that additional molecular epidemiological studies are warranted to extend these findings. [*Carcinogenesis.* **22** (2001): 593-597]

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*, in press].

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*, in press].

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]

Ścieglińska D., Widłak W., Konopka W., Poutanen M., Rahman N., Huhtaniemi I., Krawczyk Z.: ***Structure of the 5' region of the Hst70 gene transcription unit: presence of an intron and multiple transcription initiation sites.***

The rat Hst70 gene and its mouse counterpart Hsp70.2 belong to the family of Hsp70 heat shock genes and are specifically expressed in male germ cells. Previous studies regarding the structure of the 5' region of the transcription unit of these genes as well as localization of the 'cis' elements conferring their testis-specific expression gave contradictory results [Widlak, Markkula, Krawczyk, Kananen and Huhtaniemi (1995) Biochim. Biophys. Acta 1264, 191-200; Dix, Rosario-Herrle, Gotoh, Mori, Goulding, Barret and Eddy (1996) Dev. Biol. 174, 310-321]. In the present paper we solve these controversies and show that the 5' untranslated region (UTR) of the Hst70 gene contains an intron which is localized similar to that of the mouse Hsp70.2 gene. Reverse transcriptase-mediated PCR, Northern blotting and RNase protection analysis revealed that the transcription initiation of both genes starts at two main distant sites, and one of them is localized within the intron. As a result two populations of Hst70 gene transcripts with similar sizes but different 5' UTR structures can be detected in total testicular RNA. Functional analysis of the Hst70 gene promoter in transgenic mice and transient transfection assays proved that the DNA fragment of approx. 360 bp localized upstream of the ATG transcription start codon is the minimal promoter required for testis-specific expression of the HST70/chloramphenicol acetyltransferase transgene. These experiments also suggest that the expression of the gene may depend on 'cis' regulatory elements localized within exon 1 and the intron sequences. [*Biochem J.* **359** (2001): 129-137].

Małusecka E., Zborek A., Krzyżowska-Gruca S., Krawczyk Z.: ***Expression of heat shock proteins HSP70 and HSP27 in primary non-small cell lung carcinomas. An immunohistochemical study.***

The present study was undertaken to determine the expression pattern of the hsp70 and the hsp27 genes in 106 cases of non-small cell lung carcinoma (NSCLC). We have shown that in the majority of cases (95/106) the HSP70 immunoreactivity was localized both in the cytoplasm and the nuclei. We also observed an enhanced nuclear immunoreaction for HSP70 in dysplastic lesions and in stage I tumors. In the case of the HSP27 we found a positive cytoplasmic immunostaining in 70% of cases, with the highest score in squamous cell carcinoma (SCC). We noted a positive correlation between the expression level of HSP27 and HSP70. There was a correlation between Ki-67 proliferation index and nuclear HSP70 staining, but not for HSP27. No association between the HSPs and the expression of pRb p16 and p21WAF1/CIP1 and p53 was found as studied previously. An interesting and statistically significant relationship was found between the expression of cyclin D1 and high intensity of HSP27 and HSP70 immunostaining. The relation of our results concerning the

expression pattern of the HSP70 and HSP27 in NSCLC to those obtained by others for different types of primary tumors is discussed. *Anticancer Res*. **21** (2001): 1015-1021]

Rafiee M., Kanwar J.R., Berg R.W., Lehnert K., Lisowska K., Krissansen G.W.: ***Induction of systemic antitumor immunity by gene transfer of mammalian heat shock protein 70.1 into tumors in situ.***

Heat shock proteins (hsps) chaperone cytosolic peptides, forming complexes that stimulate antitumor immunity. Hsps facilitate signal 1 in the two-signal model of T-cell costimulation, whereas cell adhesion molecules such as B7.1 provide secondary (signal 2) costimulatory signals. B7.1 gene transfer into tumors in situ has been shown to eradicate small (<0.3 cm in diameter) tumors in mice, and induce systemic antitumor immunity, but is ineffective against larger tumors. We examine whether mammalian hsps, as facilitators of T-cell costimulation, also exhibit this ability, and whether simultaneously stimulating both signal 1 (hsp-facilitated antigen presentation) and signal 2 (B7.1-mediated costimulation) enhances antitumor immunity compared to that achieved with either monotherapy. Prophylactic vaccination of mice with an hsp preparation from an EL-4 lymphoma weakly retarded tumor growth, to the same extent as that achieved with a single EL-4-derived peptide (AQHPNAELL), previously shown to induce antitumor immunity establishing that a preparation of EL-4 hsp-peptide complexes has antitumor activity. Here we show that injection of rat hsp70.1 into mouse tumors in situ causes the complete eradication of tumors, and generates potent systemic antitumor immunity mediated by CD4+ and CD8+ T cells. Unexpectedly, simultaneous gene transfer of hsp70.1 and B7.1 compromised the efficacy of hsp-mediated tumor rejection--a problem which could be partially overcome by the timed delivery of hsp70.1 and B7.1. Thus, gene transfer of hsp70 into tumors can be employed to generate potent systemic antitumor immunity, but further consideration is required if this approach is to be successfully combined with immunotherapies employing other T-cell costimulators. [*Cancer Gene Ther.* **8** (2001): 974 - 981]

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]

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]

Laboratory of Molecular Diagnostics and Radioimmunology
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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:

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]

Wiench M., Wygoda Z., Gubała E., Włoch J., Lisowska K., Krassowski J., Ścieglińska D., Fiszer-Kierzkowska A., Lange D., Kula D., Zeman M., Roskosz J., Kukulska A., Krawczyk Z., Jarzab B.: ***Estimation of risk of inherited medullary thyroid carcinoma in apparent sporadic patients.***

PURPOSE: The study was undertaken to evaluate the frequency of inherited medullary thyroid carcinoma (MTC) among patients with apparent sporadic disease. A stepwise algorithm was used depending on clinical indices and the age of patient at MTC diagnosis. PATIENTS AND METHODS: One hundred sixteen patients with MTC verified by postoperative pathologic examination were subjected to genetic analysis of RET exons 10, 11, 13, 14, and 16 by means of polymerase chain reaction, restriction endonuclease digestion, and DNA sequencing. RESULTS: Among 116 apparent sporadic MTC patients, we identified eleven (9.5%) RET germline mutation carriers. Seven of these (6.0%) were found by routine analysis (exons 10 and 11). The frequency of inherited disease among patients younger than 45 years at diagnosis was 10.2% by analysis of typical mutations in exons 10 and 11. Extended genetic analysis (sequencing of exons 11, 13, 14, and 16) yielded 6.1% additional diagnoses, giving a risk of 16.3% in this age group. One previously unreported mutation in exon 11 affected codon 649 (TCG>TTG, Ser>Leu). In the true sporadic MTC patients younger than 30 years at diagnosis, frequencies of 36% and 4.5% in polymorphic variants L769L and S836S, respectively, were observed. The frequency for L769L was higher than in older patients ($P <.05$). CONCLUSION: The frequency of inherited disease among apparent sporadic medullary thyroid carcinoma patients is close to 10% in the Polish population of MTC patients. The extended analysis of all known RET proto-oncogene mutation sites is obligatory in patients younger than 45 years at diagnosis, but we also see the need to analyze the impact of rarer mutations in older patients. [*J. Clin. Oncol*. **19** (2001) 1374-1380]

Kula D., Jurecka-Tuleja B., Gubała E., Krawczyk A., Szpak S., Jarząb M.: ***Association of polymorphism of LTalpha and TNF genes with Graves' disease.***

Graves' disease (GD) is an autoimmune disease, which develops on the basis of an interaction between genetic, environmental and endogenous factors. GD is associated with some HLA genes. Closely linked with them are TNF genes (TNF and LTalpha). Their role in the pathogenesis of GD is still unclear. Two functional polymorphisms within TNF genes include a substitution of G with A in intron I of LTalpha gene and the same one at position -308 in the TNF gene promoter. We carried out a case-control study for the analysis of the contribution of TNF genes to GD in Polish patients. 156 patients with GD diagnosed by clinical data were investigated and compared to 80 healthy persons with negative familial anamnesis. Both TNF and LTalpha were analysed by PCR/Nco I RFLP. The allelic frequency of the rarer TNF2 (A) allele, was 24.7% in GD patients,

significantly higher than in healthy persons (9.3%; p<0.0001). The OR was 4.38 for this allele. The frequency of heterozygotes was 41.8% in GD, as compared to 13.6% in the control group. The allelic frequency of the rarer LTB*1 (G) allele was also significantly increased: from 21.9% in the control group to 37.2% in GD patients (p<0.01; OR 2.81). The frequency of heterozygotes was 48.7% in GD, and 28.8% in the control group. The results indicate that TNF genes may contribute to GD in the Polish population. [*Folia Histochem. Cytobiol*. **39** Suppl 2 (2001) 77-78]

Czarnocka B., Pastuszko D., Janota-Bzowski M., Weetman A.P., Watson P.F., Kemp E.H., McIntosch R.S., Asghar M.S., Jarząb B., Gubała E., Włoch J., Lange D.: ***Is there loss or qualitative changes in the expression of thyroid peroxidase protein in thyroid epithelial cancer?***

There is disagreement concerning the expression of thyroid peroxidase (TPO) in thyroid cancer, some studies finding qualitative as well as quantitative differences compared to normal tissue. To investigate TPO protein expression and its antigenic properties, TPO was captured from a solubilizate of thyroid microsomes by a panel of murine anti-TPO monoclonal antibodies and detected with a panel of anti-human TPO IgGkappa Fab. TPO protein expression in 30 samples of malignant thyroid tissue was compared with TPO from adjacent normal tissues. Virtual absence of TPO expression was observed in 8 cases. In the remaining 22 malignant thyroid tumours the TPO protein level varied considerably from normal to nearly absent when compared to normal thyroid tissue or tissues from patients with Graves' disease (range less than 0.5 to more than 12.5 microg mg(-1) of protein). When expressed TPO displayed similar epitopes, to that of TPO from Graves' disease tissue. The results obtained by the TPO capturing method were confirmed by SDS-PAGE and Western blot analysis with both microsomes and their solubilizates. The present results show that in about two-thirds of differentiated thyroid carcinomas, TPO protein is expressed, albeit to a more variable extent than normal; when present, TPO in malignant tissues is immunologically normal. [*Brit. J. Can*. **85** (2001) 875-880]

Jarząb B, Włoch J, Wiench M.: ***Molecular changes in thyroid neoplasia***.

All authors integrating the known facts into a model of thyroid carcinogenesis concur that two main histotypes of thyroid cancer exhibit different routes of molecular development. RET rearrangements are an initiating event in papillary carcinoma, and simultaneously the most characteristic mutation for this type of cancer. They are followed by further, not well recognized, mutations. RAS mutations are regarded as a crucial event in the development of follicular tumors already at the adenoma step, while in papillary cancer they belong to the spectrum of secondary mutations, enabling tumor progression. Aberrant DNA methylation, causing loss of P16 tumor supressor gene, may be a common event in both types of cancer. Aneuploidy is seen much more frequently in follicular than in papillary cancer, which also exhibits a low rate for loss of heterozygosity and microsatellite instability. Mutations of the P53 tumor supressor gene are a common feature of undifferentiated thyroid cancers and could be responsible for their aggressive phenotype. RET rearrangements have been proposed as identifying fingerprints for irradiation induced thyroid cancer in children. Our own data speak against this hypothesis. We noted a high frequency of RET/PTC3 mutations in a group of Polish children with papillary thyroid carcinoma, regarded as sporadic cancer. [*Folia Histochem Cytobiol*. **39** Suppl 2 (2001) 26-27]

Wiench M., Wygoda Z., Gubala E., Wloch J., Oczko M., Jarzab B.: ***The genetic background of medullary thyroid carcinoma in young patients***

The study was undertaken to evaluate the frequency of RET polymorphisms at codons 769 and 836 in young medullary thyroid carcinoma (MTC) patients in whom the presence of a known germline mutation has been excluded. 40 patients aged 10-29 were subjected to genetic analysis of RET exons 10, 11, 13, 14 and 16 and compared to 140 older patients. The hereditary component occurred to be very high in young MTC patients: 57% carry the germline mutation, other 28% exhibit at least one rare polymorphic variant of RET. The observed allelic frequencies were 38% for polymorphic variant L769CTG and 6% for variant S836AGT. The results were significantly higher than those obtained in the group of older patients: 20% and 1% for L769CTG and S836AGT, respectively. Our results speak in favour that the polymorphism in RET codon 769 and 836 may also be a factor predisposing to the development of MTC in young age. [*Folia Histochem Cytobiol*. **39** Suppl 2 (2001) 163-164]

Lange D., Ferenc T., Niewiadomska H., Włoch J., Turska M., Burkacka J., Kula D., Lewinski A., Jarząb B.: ***Prognostic significance of selected oncogene and suppressor gene expression in follicular thyroid carcinoma.***

Oncogene and suppressor gene expression (cyclin D, p21WAF1, nm23-H1, Rb1, p16INK4A, and p53) was evaluated in 23 follicular thyroid carcinomas diagnosed in 20 women and 3 men operated or reoperated in

Institute of Oncology in Gliwice in years 1992-1999. Positive reaction with p16INK4A, Rb1 and cyclin D1 antibodies was observed in all tumors, with nm23-H1 in 22 cases. The presence of p21WAF1 was stated in 8 cases (34.8%) and p53 in 7 cases (30.4%). A simultaneous presence of expression of p53 and lack of expression of p21WAF1 was stated three times and in two cases were accompanied by distant metastases. This pattern of expression was only rarely observed in minimally invasive follicular cancer. The prognostic significance of simultaneous immunohistochemical analysis of p53 and p21WAF1 in follicular thyroid carcinoma is suggested and has to be proved in further studies. [*Wiad. Lek.* **54** Suppl 1 (2001) 72-78, in Polish]

Wiench M., Włoch J., Oczko M., Gubała E., Jarząb B.: ***Rearrangement of the RET gene in papillary thyroid carcinoma***

The RET/PTC oncogenes, activated forms of the RET protooncogene, almost exclusively found in papillary thyroid carcinoma (PTC). What is more, the targeted expression of RET/PTC in mice leads to the development of thyroid tumors very similar to human PTCs. In all RET/PTC types the RET tyrosine kinase domain is fused to the N-terminus of ubiquitously expressed genes that is capable of ligand-independent dimerization. The majority of RET/PTC identified consists of two types which results from the inversion of chromosome 10: RET/PTC1 and RET/PTC3. The prevalence of RET/PTC in papillary thyroid carcinomas of thyroid varies widely from a few to about 80% with the highest frequency in tumors arising in children after ionizing radiation. In Polish population the frequency of RET rearrangements in papillary cancers is 27%, although, it was reported to be twice higher in young patients (50% in patients younger than 21 at operation). Correlation with clinical outcome as well as prognostic value of RET/PTC is controversial. Some authors suggest that it predicts metastases, others found rearranged RET in more favourable, slow growing tumors. RET/PTC3 seems to be associated with solid/follicular variant PTC and short latency period (it is found more frequently in children) whereas RET/PTC1- with classic PTC variant and long latency. [*Wiad. Lek.* **54** Suppl 1 (2001) 64-71, in Polish]

Wiench M., Kwaśniewski M., Gubała E., Wygoda Z., Pawlaczek A., Oczko M., Jarząb B.: ***Proto-oncogene RET somatic mutations in medullary thyroid carcinoma***.

Somatic mutations of the RET protooncogene are present in 23-68% cases of sporadic medullary thyroid carcinoma (MTC). The aim of the study was to introduce the RET somatic mutations analysis in tumor tissue as well as to evaluate their types and frequencies in postoperative specimens of MTC patients treated in the Center of Oncology in Gliwice. MATERIAL: 14 tumor tissues obtained from sporadic MTC patients and two control groups--six and four specimens from patients with MEN 2A and MEN 2B syndrome respectively. METHODS: Tumor tissue DNA isolation followed by PCR amplification of RET exons 10, 11, 13, 14, 16 and automated, fluorescent sequencing of PCR products. We identified somatic mutation ATG > ACG in codon 918, exon 16 in 7 of 14 (50%) of analyzed sporadic MTC cases. We also found one deletion/insertion mutation in RET exon 11 that encompasses cysteine codon 634 and has not been published so far. The types and frequencies of found RET gene mutations were similar to previously reported. The analysis of RET somatic mutations supports the differentiation between the sporadic and inherited MTC. The presence of somatic mutation and its simultaneous absence in the germline proves sporadic type of cancer. [*Wiad. Lek.* **54** Suppl 1 (2001) 415-421, in Polish]

Gubała E., Handkiewicz-Junak D., Zeman M., Chmielik E., Wiench M., Jarząb B.: ***Thyroglobulin RT-PCR method for detection of lymph node metastases during the course of differentiated thyroid cancers.***

In patients with a suspicion of recurrence of differentiated thyroid cancer with metastases to lymph nodes, detection of thyroglobulin (Tg) mRNA in fine needle biopsy material may support the interpretation of classic cytological examination in cases where it fails to detect lymph node involvement early enough. AIM: Prospective study of thyroglobulin mRNA detection in neck lymph nodes in patients with suspected differentiated thyroid cancer (DTC) metastases. MATERIAL: 70 nodes from 60 patients with suspected DTC recurrence were investigated. Patients with suspicion of lymph node metastases of other types of cancer were included as a control group. Thyroglobulin RT-PCR was conducted in residual material left after preparation of cytological smears from fine needle biopsy specimens. Primers spanning exons 3-5 were used with 39 cycles of PCR. RNA isolation control and cDNA amplification were carried out using GADPH starters. RESULTS: Classical cytology confirmed nodal involvement in 22 of DTC patients, RT-PCR Tg was positive in 20 of them (91%). Among 48 patients with a suspicion of DTC recurrence and negative cytology, Tg mRNA was found twice. One positive RT-PCR result was confirmed by repeated cytology conducted 4-6 months later and followed by surgery. No positive result of RT-PCR was obtained with other head and neck malignancies. The overall specificity was estimated with 98%. CONCLUSIONS: RT-PCR Tg shows sufficient specificity to be applied in further studies estimating its usefulness in fine needle biopsy for early detection of lymph node metastases in differentiated thyroid cancer. [*Wiad. Lek.* **54** Suppl 1 (2001) 349-356, in Polish]

Wygoda Z., Włoch J., Gubała E., Wiench M., Jarząb B.: ***Results of treating medullary thyroid carcinoma: the differences between sporadic and inherited forms.***
Medullary thyroid carcinoma (MTC) can be divided into two subgroups: sporadic or inherited. Hereditary form of MTC is often believed to be form with better prognosis than sporadic one. In this study the differences in MTC prognosis in Polish population of patients was analyzed. The group of 169 patients with MTC was examined. Hereditary cancer was stated in 48 (28%) patients. The median age of disease onset was 41 years (from 7 to 71 years). Genetic examination of RET protooncogene was performed in all patients. The calcitonin and CEA serum level analysis and radiological and radioisotopic examinations were used for monitoring of the disease course. Nineteen cases of MEN 2A syndrome, 11 cases of MEN 2B one and 18 cases of non classified familial MTC were recognized among patients with inherited MTC. Significantly lower age of disease onset in inherited MTC than in sporadic one was observed (27 years vs. 43.7 years, $p < 0.001$). Local or nodal recurrence was observed in 22 (13%) patients, distant metastases were stated in 21 (12%) patients. Basal or stimulated serum calcitonin level was increased in 85 (50%) patients. No significant differences between sporadic and inherited disease were observed. Eight patients died during observation, including 3 patients with sporadic MTC and 5 patients with inherited MTC. The updated 10-year survival rate was 97% in patients with sporadic MTC; in hereditary MTC it was about 20% worse. The complications related to the presence of adrenal tumors were the main reason for death in MEN2 and no significant differences in the course of MTC itself were observed. [*Wiad. Lek.* **54** Suppl 1 (2001) 422-431, in Polish]

Włoch J., Wygoda Z., Wiench M., Gubała E., Kula D., Oczko M., Lange D., Jarząb B.: ***Consequences of clinical genetic analysis of RET proto-oncogene***
Preliminary results of treatment of inherited medullary thyroid carcinoma, diagnosed primarily with genetic analysis of mutation of protooncogene RET are presented. Among 16 carriers of mutation identical with mutation diagnosed earlier in proband, there were 4 patients with clinically obvious medullary thyroid carcinoma and 12 asymptomatic carriers. In all patients, in whom calcitonin level was increased preoperatively, its normalization was obtained. The paper summarizes these aspects of cooperation between geneticians and physicians in which diagnostic results influence clinical decisions (indication and time of thyroid and lymph nodes surgery and it's spectrum, range of diagnostic procedures towards pheochromocytoma and parathyroid hyperplasia in relation to the found mutation). [*Wiad. Lek.* **54** Suppl 1 (2001) 406-414, in Polish]

Matuszewska G., Roskosz J., Włoch J., Jurecka-Tuleja B., Hasse-Lazar K., Kowalczyk P., Jarząb B.: ***Evaluation of effects of L-thyroxine therapy in differentiated thyroid carcinoma on the cardiovascular system - prospective study***
Patients with differentiated thyroid carcinoma are treated by thyreoidectomy, followed by radioiodine treatment. A life-time suppressive therapy with L-thyroxine is also indicated. However, it may cause cardiovascular side effects. The aim of the study was a prospective evaluation of the left ventricle hypertrophy in patients treated with suppressive doses of thyroxine. A significant rise in left ventricular mass and mass index was noted during the first year of therapy and could be prevented by a simultaneous treatment with low doses of bisoprolol. [*Wiad. Lek.* **54** Suppl 1 (2001) 406-414, in Polish]

Czernik E., Deja R., Gubała E., Kukulska A., Turska M., Dabrowska-Czuba E., Bartnikowa W., Jarzab B.: ***Comparison of efficacy of various methods of thyroglobulin measurements in differentiated thyroid cancer***
Monitoring patients with differentiated thyroid carcinoma (DTC) by thyroglobulin (Tg) measurements requires selecting optimal methods used for detection of this marker. An increase the thyroglobulin concentration in serum is a predictor of tumor recurrence. All serum thyroglobulin assays can be falsified by presence of Tg autoantibodies, which are present in approximately 20% of DTC patients. The aim of this study was a comparison of the clinical utility of two different methods for determining serum Tg concentration in monitoring patients with DTC during thyroxine treatment. Tg concentration was measured in serum samples of 1530 patients with DTC during replacement thyroid hormone using two methods: fluoroimmunometric assay (IFMA) Wallac Delfia Thyroglobulin and immunoradiometric assay (IRMA) Brahms DYNOtest Tg-S. 1847 values of Tg concentration and recovery test detected between 1992 and 1995 years using IFMA methods and 1187 values of Tg and thyroglobulin autoantibodies concentration measured in 2000 year using IRMA methods were also included. The correlation between Tg values in all patients group wasn't good ($r = 0.83$; $p < 0.05$), but when we excluded patients with incorrect recovery test determined by IRMA, the correlation factor was higher ($r = 0.94$; $p < 0.05$). The estimation of Tg recovery test obtained in IRMA assay eliminated from monitoring only 3% patients with DTC, when IFMA assay excluded nearly 1/5 patients, whereas the estimation Tg autoantibodies

7% from all patients. The IRMA method is the most resistant to interference and allows to monitor a reliably greater group of patients with DTC during thyroxine treatment. [*Wiad. Lek.* **54** Suppl 1 (2001) 339-348, in Polish]

Kukulska A., Gubała E., Turska M., Handkiewicz-Junak D., Czernik E., Deja R., Blamek S., Szpak S., Jarząb B.: ***Prospective analysis of criteria for interpreting measurements of thyroglobulin serum concentration during conditions of endogenous TSH stimulation in serum of patients with differentiated thyroid carcinoma***

The aim of this study was the assessment of diagnostic value of thyroglobulin serum measurement in patients with DTC during endogenous TSH stimulation. Thyroglobulin was measured by immunofluorometric method (Delfia-Wallac) in patients after combined surgery and I131 ablation. Predictive values for two threshold levels 10 and 30 ng/ml were compared. At 5 years follow up it has been demonstrated, that Tg values higher than 10 ng/ml were the true signals of DTC relapse only in 46% patients. Tg values higher than 30 ng/ml were associated with disease progression in 65% of patients. Thus, we accept Tg concentration of 30 ng/ml measured during endogenous TSH stimulation as a good cut-off limit for the detection of DTC progression. Reduction of this threshold up to 10 ng/ml is associated with the increased risk of false positive results. [*Wiad. Lek.* **54** Suppl 1 (2001) 332-338, in Polish]

Handkiewicz-Junak D., Roskosz J., Turska M., Wygoda Z., Jarząb B.: ***Use of 13-cis retinoic acid for treatment of advanced differentiated thyroid cancer***

Retinoids, a large group of compounds structurally related to vitamin A, are able to induce redifferentiation of thyroid cancer cells. The aim of the study is to present our early results of retinoids redifferentiation therapy of thyroid cancer patients. In 15 patients with advanced thyroid cancer, whose cancer foci did not concentrate radioiodine, 13-cis retinoic acid (Roaccutan) was given for 6 weeks before radioiodine treatment. Radioiodine therapy was performed under exogenous TSH stimulation (Thyrogen). Three patients were treated twice. The planned retinoid dose was delivered to 11 patients. In the other four patients the reduction of retinoids dose was necessary due to severe side effects. In post-therapeutic scintigraphy radioiodine uptake was visible in two out of seven patients (29%) with lung metastases, in 5 out of 9 (56%) with locoregional disease and in two with bone metastases. On the whole, in 50% of patients reinduction of radioiodine uptake was visible, however, in most patients only a very discrete one. Thyroglobulin concentration before and after retinoids therapy did not differ significantly. CONCLUSIONS: In a subgroup of patients 13-cis retinoic acid can induce radioiodine uptake, however, prospective studies in larger groups of patients are necessary to prove its clinical application. [*Wiad. Lek.* **54** Suppl 1 (2001) 301-306, in Polish]

Roskosz J., Handkiewicz-Junak D., Turska M., Wygoda Z., Jurecka-Tuleja B., Jarząb B.: ***Use of recombinant human TSH for stimulation of iodine radioisotope uptake in metastases of thyroid cancer during therapy with 131I***

The diagnostics with the use of recombinant human TSH for the follow-up of differentiated thyroid carcinoma (DTC) has been already approved. In more than 400 diagnostic scans, rhTSH proved to be effective in promoting 131I uptake in thyroid remnants and DTC metastases in patients receiving suppressive doses of thyroxine. However, information about its application in radioiodine treatment of DTC are scarce, especially with respect to patients with metastatic disease. In this review we have described our own results obtained during rhTSH aided radioiodine treatment of 42 patients with advanced DTC with reference to current literature data about diagnostic and therapeutic application of rhTSH. [*Wiad. Lek.* **54** Suppl 1 (2001) 289-296, in Polish]

Gawkowska-Suwińska M., Turska M., Roskosz J., Puch Z., Jurecka-Tuleja B., Handkiewicz-Junak D., Wygoda Z., Jarząb B.: ***Early evaluation of treatment effectiveness using 131I iodine radiotherapy in patients with differentiated thyroid cancer***

This paper presents the preliminary results of a prospective randomized trial on early effectiveness of 30 mCi versus 60 mCi for ablation of thyroid remnants in patients with WDTC after total thyroidectomy. Since April 1998 to January 2000, 220 patients with papillary thyroid cancer in stage T1b-3, N0-x, M0 had entered the study. 106 patients received 60 mCi and 114 received 30 mCi as the first ablation dose. The subject for the analysis was the uptake over the neck, post-therapeutic whole body scintigraphy and Tg level 6 months after ablation. The early effectiveness of ablation was estimated using a 5-degree scale: 0--very good effect, 1--good effect, 2--dubious effect-required repetition of WBS and Tg assessment in 6-12 months, 3--insufficient ablation—required repetition of radioiodine treatment, 4--for evident dissemination or local recurrence. RESULTS: Grades 0 were obtained in 29 (53%) after 30 mCi (group I) and in 38 patients (86%) after 60 mCi (group II). Grades 1 were

obtained in group I in 15 patients (28%) and in 4 patients (9%) of group II. Grades 2 were obtained in group I in 9 patients (17%) and in group II in 1 (2.3%). Grade 3 was obtained only in 1 (2%) patient after 30 mCi. Grade 4 was obtained in one patient after 60 mCi (2.3%). The difference in uptake over the neck in the two groups was statistically significant ($p < 0.05$), although the differences in early effectiveness between the both groups according to the 5-degree scale were on the borderline of significance ($p = 0.075$). There was a correlation between uptake before and after ablation in 30 mCi group, which was not seen present in 60 mCi group. CONCLUSION: For the ablation of thyroid remnants 60 mCi should be considered as a standard dose. [*Wiad. Lek.* **54** Suppl 1 (2001) 278-288, in Polish]

Jarząb B.: ***Indications for adjuvant therapy of thyroid cancer with radioiodine therapy***
Indications for complementary radioiodine therapy of differentiated thyroid carcinoma are based on its potential to sterilize micrometastases and to ablate thyroid remnants. A short summary of the assumptions leading to the combination of total thyroidectomy with complementary radioiodine treatment is given in the paper. We also present the preliminary results of the evaluation of nearly 800 patients with differentiated thyroid carcinoma treated surgically in various centres and referred to our department for radioiodine therapy. Total 10-year survival was significantly higher in patients in whom complementary radioiodine therapy had been introduced after total or less than total thyroidectomy. The upper limit of postoperative 24 h thyroid uptake of 20% before radioiodine therapy was revealed as a good criterium for the optimal long-term effects. However, a gradual decrease of postoperative 131I uptake has been observed, thus, this upper limit is probably to be lowered in future. [*Wiad. Lek.* **54** Suppl 1 (2001) 254-265, in Polish]

Handkiewicz-Junak D., Wygoda Z., Włoch J., Jarzab B.: ***Possibilities and limitations of intraoperative isotope detection for thyroid carcinoma***
Intraoperative probes become increasingly important in the surgical management of cancer. Attempts with gamma probe guided surgery to improve the completeness of surgical excision of radioiodine avid tissues in thyroid cancer have been performed through several decades. The first Polish results by Pomorski et al. have shown that gamma probe guided surgery after preoperative dose of 131I have allowed locating and increasing the completeness of thyroid excision. These results have been substantiated by other authors. However, in the evaluation of intraoperative gamma probe localization of 131I avid tissues one should remember of the limitations of the method. The article begins with a discussion of the statistical limitations of the radiation detection and of the key performance parameters that characterize detectors. Later on we continue with the description of specific aspects concerning gamma probe guided surgery in thyroid cancer. [*Wiad. Lek.* **54** Suppl 1 (2001) 246-252, in Polish]

Kowalczyk P., Roskosz J., Jurecka-Tuleja B., Gubała E., Czernik E., Jarząb B.: ***L-thyroxine therapy in differentiated thyroid carcinoma: criteria for evaluation of TSH suppression***
Life-time L-thyroxine therapy is obligatory in patients treated for differentiated thyroid carcinoma (DTC) in order to suppress serum TSH. The rationale for that is the TSH stimulation of follicular cells' growth and the presence of TSH receptors on DTC cells. Nevertheless, the exact criteria for TSH suppression in DTC are not specified and are a matter of discussion, stimulated by the recent progress in the evaluation of thyroxine side effects on bone and heart. The aim of the study was the optimalization of the reference range for TSH suppression in DTC patients in order to minimalize the risk of iatrogenic thyrotoxicosis. One hundred and twenty nine patients were randomly chosen among patients treated radically for DTC (116 females and 13 males). Basal and TRH stimulated TSH level, FT4 and FT3 serum level were estimated by microimmunoenzymatic method, while SHBG was estimated by immunofluorimetry. Full suppression (basal TSH < 0.05) was obtained in 64 patients (49%), submaximal suppression (TSH between 0.1 and 0.3 mU/l) was observed in 21 patients (16%). In 29 patients (22%) no suppression was obtained by the applied dose of thyroxine. The risk of iatrogenic hyperthyreosis, as judged by the increase of FT3 or SHBG, was found to be 38% in patients with full suppression and only 5% in patients with submaximal suppression ($p < 0.05$). CONCLUSION: 1. Suppression of TSH secretion was achieved in 80% of patients with differentiated thyroid carcinoma. The control of TSH level must be controlled every 3 months in 5 first years of therapy. 2. The optimal serum TSH level for L-thyroxine therapy in asymptomatic patients after radical treatment of differentiated thyroid carcinoma ranges between 0.1 to 0.3 mU/L. This range ensures the expected suppression of TSH with only minimal risk of iatrogenic hyperthyreosis. [*Wiad. Lek.* **54** (2001) 268-276, in Polish]

Kowalczyk P, Sielanczyk A, Nowak J, Matuszewska G, Roskosz J, Czernik E, Gubała E, Jarzab B. ***Effects of L-thyroxine suppressive therapy on cardiac mass in patients with differentiated thyroid cancer***

Patients with differentiated thyroid carcinoma (DTC) receive a life time l-thyroxine therapy in suppressive doses and may exhibit signs of cardiac hypertrophy. The aim of the study was to analyze the left ventricle mass parameters by echocardiography in patients treated with suppressive doses of thyroxine and to relate them to the possible occurrence of cardiac arrhythmias. Ninety four patients aged 19-70 years treated chronically with l-thyroxine were randomly chosen from the population of patients with DTC without concomitant diseases of circulatory system. They were divided into two subgroups according to the length of thyroxine therapy (< 60 months and > or = 60 months). Control group consisted of 41 healthy volunteers, aged 22-73 years. Heart muscle dimensions were measured by echocardiography. Left ventricle mass (LVM) and mass index (LVMI) was calculated. Electrocardiography according to Holter was carried out in 57 patients. The results of echocardiography in the whole group of patients did not differ significantly from the control group, although a tendency towards higher dimensions of the left ventricle was observed. No correlation of hormonal parameters, or thyroxine dose, with LVM or results of Holters ecg was noted. When patients were subdivided into two groups, according to the duration of therapy, significantly higher values of LVM (215 +/- 64 g versus 186 +/- 55; $p < 0.05$) and LVMI (114 +/- 31 g/m versus 102 +/- 23 g/m; $p < 0.05$) were observed in patients treated > or = 60 months in comparison to the control group. When results of Holter's ecg in patients with increased LVMI were analyzed, cardiac rhythm disturbances were stated in 50% of them, but most were of minor clinical relevance. Suppressive l-thyroxine therapy does not induce significant left heart hypertrophy during the first 5 years of treatment. Patients treated through a longer period of time should be controlled by echocardiography because of the increasing risk of the left ventricle hypertrophy and arrhythmia. [*Pol Arch. Med. Wewn.* **105** (2001) 123-130, in Polish].

Lange D., Sporny S., Sygut J., Kula D., Burkacka J., Jarząb M., Kulig A., Jarząb B.: ***Differential criteria between papillary and follicular thyroid carcinoma-initial conclusions from a multicenter trial***

Histopathological diagnosis of thyroid cancer is difficult and requires much experience. Pathologists have to know many histopathological variants and be aware of the current diagnostic criteria. The aim of the study was to unify criteria applied all over the country and compare whether the accuracy of diagnosis has changed in the course of the last fifteen years. In a multicenter trial, 36 pathologists from 25 centers reevaluated 232 thyroid tumors operated between 1985-1998. The reference diagnosis was given on the basis of evaluation made by four experienced pathologists. The two-step analysis was performed. At first, the accuracy of the diagnosis of malignant neoplasm was evaluated. Then, the accuracy of the diagnosis of the cancer histotype was analyzed, with estimation of kappa coefficients and their asymptomatic standard error. Comparison of primary and reference diagnoses revealed statistically significant differences--in 17% of cases the primary diagnosis of cancer was not confirmed by experienced pathologists. Kappa coefficient for the diagnosis of cancer histotype was 0.53 + 0.06. On the contrary, the diagnoses made by the participants of the trial did not differ significantly from the reference ones. Kappa coefficient for the diagnosis of cancer histotype was significantly higher than for primary diagnoses with 0.63 +/- 0.10 ($p < 0.001$). The first results of the multicenter trial indicated that the most frequent diagnostic error made at primary diagnosis was the overdiagnosis of follicular thyroid carcinoma. Thus, a summary of strict criteria for papillary and follicular thyroid carcinoma is also given. [*Wiad. Lek.* **54** Suppl 1 (2001) 42-53, in Polish]