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Centre of Excellence

Division of Experimental Oncology

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

http://cd.io.gliwice.pl

Scientific Report 2005-2006

General Information. Maria Skłodowska-Curie Memorial Cancer Center and Institute of Oncology in Gliwice is an European Comprehensive Cancer Center and one of the largest clinical institutions in Poland. The Division of Experimental Oncology is involved in many aspects of basic, translational, and clinical research in oncology since 1950. The Division comprises the Departments of Tumor Biology, Molecular Biology, Experimental and Clinical Radiobiology, Epidemiology, Medical Physics and Nuclear Medicine. Its research staff is well-trained, experienced and is well recognized on the Polish and international research maps. In 2004, the Division of Experimental Oncology was awarded the title of "National Center of Excellence" by the Polish Ministry of Science.

Research staff. The staff of the Center of Excellence numbers about 60 people with university education and includes: 8 full professors, 4 associate professors and 25 Ph.D. researchers. The scientists are proficient in modern molecular and cell biology methods. Particularly strong areas of expertise are gene cloning, gene structure and function, transgenic mice, DNA microarrays, RT-PCR, immunohistochemistry, confocal microscopy, polymorphic genes involved in metabolism of xenobiotics and DNA repair. They have close and efficient collaboration with physicians from clinical departments of parent Institute and other local medical institutions.

Research interest. Research activity at the Center is devoted to translational research in experimental oncology and mainly pertains to molecular diagnostics and experimental therapies of cancer. Specifically, research activities are focused on: (i) genetic risk factor in cancer predisposition, (ii) novel prognostic and predictive markers for cancer therapy; (iii) genomic and proteomic signatures for cancer identification and classification; (iv) novel diagnostic tools for monitoring therapy; (v) pharmacology and biology of novel anticancer drugs; (vi) novel vectors and strategies of drug delivery.

Collaboration. Ongoing collaborative projects with prestigious scientific centers in Europe, USA and Canada, including: Deutsches Krebsforschungszentrum, Heidelberg (Germany); IARC, Lyon (France); Columbia University, New York (USA); Turku University (Finland); the National Cancer Institute, NIH, Bethesda (USA); University of Texas Southwestern Medical Center, Dallas (USA); Thomas Jefferson University, Philadelphia (USA); the Karolinska Institutet, Stockholm, (Sweden); the Institute of Industrial Medicine, Milan (Italy); Laval University, Quebec (Canada).
Research groups cooperate actively with a team of mathematicians and computer scientists from the Silesian University of Technology in Gliwice, who specialize in biostatistics and mathematical analyses of biological phenomena. The Center's staff is involved in graduate programs offered by three Silesian institutions of higher education: the Silesian University (Faculties of Biology and Physics), the Silesian Medical Academy and the Silesian University of Technology.

Relevant web pages:
Annual conference – Gliwice Scientific Meetings: http://gsn.io.gliwice.pl/

Department of Tumor Biology

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RESEARCH PROFILE

Molecular genetics and molecular epidemiology of cancer, mechanisms controlling stability of the genome, mechanisms of cellular senescence, molecular mechanisms of cancer cell migration, regulation and function of heat shock genes in carcinogenesis and differentiation, search for molecular and immunohistochemical tumor markers, study of novel anticancer drugs and therapies.

CURRENT RESEARCH ACTIVITIES

Identification of genetic markers of lung cancer risk in women. We perform search for genetic markers, which may be useful in determining the individual risk of cancer. Using DNA samples isolated from female lung cancer patients and healthy controls, we genotype the polymorphisms of genes coding for cytoprotection proteins. With this case–control approach we analyze dozens of genetic markers (polymorphisms) using PCR-RFLP or DNA sequencing methods. We also search for germline p53 mutation in female lung cancer patients and breast cancer patients with strong family history of cancer. Project is held in cooperation with the Department of Cancer Epidemiology, Cancer Center and Institute of Oncology, Kraków. [M.Rusin, rusinm@rocketmail.com; D. Butkiewicz, dorotab@rocketmail.com]

Molecular profile of hereditary and sporadic breast cancer. About 10% of breast cancer cases develop on the background of hereditary predisposition. Among known predisposing mutations are those in BRCA1, BRCA2, p53 genes and probably also mutations in CHEK2, NOD1, NBS1 genes. Large number of familial breast cancer cases have unknown genetic background; those are called BRCAx cases. We compared gene expression profile of hereditary breast cancer (BRCA1 mutation-linked and BRCAx cases) and sporadic breast cancer. Interestingly, we found, that BRCAx tumors have more distinctive gene expression profile from sporadic tumors than BRCA1-linked ones. Currently we validate usefulness of several selected genes/proteins as molecular markers of BRCA1 mutation-linked breast cancer. When positively validated such markers would be used to pre-screen breast cancer patients for possible BRCA1 mutation in cases with non-informative familial history of cancer. [K. Lisowska, kasial@io.gliwice.pl]

Molecular profile of ovarian cancer. Early stage ovarian cancer is characterized by asymptomatic development. Due to the lack of reliable biochemical tests and other diagnostic procedures suitable for screening and early detection, ovarian cancer is usually diagnosed at an advanced stage. Standard therapy for advanced stage ovarian cancer encompasses surgery aimed to maximal possible cytoreduction and adjuvant chemotherapy. Modern chemotherapeutic regimens are based on platinum compounds and taxanes. Ovarian cancer usually responds well to these therapies, however the

development of drug resistance in many cases is the major obstacle limiting success rate. In a long run the aim of our study is to determine gene expression profile (gene signature) which is correlated with chemoresistance against different types of chemotherapy. We look for genes/proteins that could be used for prediction of individual patient response to the therapy. For better understanding the biology of ovarian cancer we are analyzing the main sources of variability and their impact on gene expression profiles in ovarian cancer. We also investigate the correlation between hereditary mutations in BRCA1 gene as well as somatic mutations in p53 gene and changes in global gene expression profile. Project is held in cooperation with the Department of Pathology, Cancer Center and Institute of Oncology, Warszawa. [K. Lisowska, kasial@io.gliwice.pl]

Identification of predictive markers in prostate cancer radiotherapy. Implementation of conformal and intensity modulated radiation therapy enables accurate delivery of radiation dose in the prostate and diminishing risk of side effects. Using DNA microarray analysis we started the project aimed to compare the expression profiles of groups of patients with different response to radiotherapy treatment: responders vs. nonresponders. Expression of selected genes, which correlate with response to treatment, will be validated on gene and protein level. A panel of potential predictive marker genes will be also analyzed by QRT-PCR technique in population of cancer cells isolated by laser-captured microdissection. We also study immunohstochemical markers relevant to radiation response in prostate cancer biopsies. Clinical application of such markers will enable more precise tailoring of therapy.[E. Małusecka, maluseck@io.gliwice.pl]

Comparison of expression profile of stromal cells in normal and cancerous prostate tissue. Several human cancers have been shown to induce a stromal reaction as a component of carcinoma progression, however the extent to which stroma regulates the biology of tumorigenesis is not fully understood. There are reports that stromal component in prostate cancer is different from normal prostate gland stroma. Our study is directed towards identification of specific markers of reactive stroma, which may be used for better prediction of the rate of cancer progression or the possibility of recurrence. Stromal cells of normal prostate tissue and prostate cancer are isolated by laser-captured microdissection. After RNA isolation the expression analysis will be performed using QRTPCR and/or microarray analysis. [A. Fiszer-Kierzkowska, anna.fiszer@plusnet.pl]

Application of laser-assisted microdissection for gene expression profiling in thyroid cancer. The project of gene expression profiling in papillary thyroid cancer is held in cooperation with Nuclear Medicine and Endocrine Oncology Department. Pure cell populations (thyreocytes, stromal cells, endothelial cells and inflammatory cells) are isolated with laser microdissection technique. Compared to RNA from bulk material now even slight differences in gene expression become detectable by quantitative RT-PCR or DNA microarrays. [A. Rusin, arusin@io.gliwice.pl; A. Zborek, azborek@io.gliwice.pl]

Molecular and functional characterization of genes and proteins involved in maintenance of genomic stability and regulation of cellular senescence. Maintenance of genomic stability protects against cancer and delays the appearance of aging. The cellular senescence that is a significant anticancer mechanism contributes to many signs of aging. The maintenance of genomic stability and cell cycle regulation are tightly coordinated. We study many aspects of this regulation. The group of genes and proteins studied by us include: RecQ helicases (WRN, BLM, RTS) associated with human cancer prone syndromes, SIRT1 deacetylase that regulates lifespan of many model organisms, BRCA1, which is a major tumor suppressor gene mutated in familial breast and ovarian cancer syndrome and PML protein positively regulating many anticancer and antiviral cellular responses. Project is held in cooperation with the Laboratory of Human Carcinogenesis, NCI, NIH, Bethesda, MD, USA. [M. Rusin, rusinm@rocketmail.com; D. Butkiewicz, dorotab@rocketmail.com]

The role of neuropilins and semaphorins in cancer cells migration. The aim of the current study is to determine how silencing of the neuropilin-1 (NRP1) gene by siRNA influences cell migration and adhesion. The diversity of NRP1 complexes, including other receptors such as plexins, VEGFR2 or CAMs makes input for multiplicity of cell response and is dependent on their ligands: semaphorin

class 3 or VEGF. Highly glycosylated NRP-1 overexpression in invasive prostate and melanoma cancer cells can affect migration and cell-specific adhesion. Knowledge of involvement of NRP1 in tumor progression via processes such as angiogenesis, cell survival and migration is the goal of the present study. We are also studying the usefulness of determination of NRP expression pattern for clinical applications. [A. Mazurek, amazurek@io.gliwice.pl, Z. Krawczyk, krawczyk@io.gliwice.pl].

The function of HSP70 genes (mainly human HSP70i and HSPA2 genes) in cancer cells and inflammation. Stress-inducible protein HSP70i is frequently constitutively expressed at high levels in primary tumors. Moreover, we found that tumor cells as well as primary tumor tissue can express the HSPA2 gene primarily defined as testis-specific Hsp70-related gene. Our main interest is to determine the functional differences (mainly cytoprotective and antiapoptotic role) between inducible HSP70 and spermatocyte-specific HSPA2 proteins in cancer cells. We are also studying the influence of the overexpression of these genes on the mitotic spindle and aneuploidy of cancer cells treated with mitotic toxins and hyperthermia. Our another interest is to determine the role of HSPs (mainly HSP70 and HSP25) in rat liver in modulation of inflammatory reaction induced by various hepatotoxicants. Using the immunohistochemical methods we study the expression pattern of these proteins in hepatocytes surrounding the areas of inflammation. We are interested in the mechanism of induction of HSPs expression by macrophages ($Ed1^+$, $ED2^+$). [D. Ścieglińska; dorotas@io.gliwice.pl, A. Rusin, arusin@io.gliwice.pl; A. Zborek, azborek@io.gliwice.pl]

Novel genistein derivatives as anticancer cytostatic drugs and radiosensitizers. Flavonoids are known to enhance radiosensitivity due to inhibition of the cell cycle progression in the phase G2/M. New genistein derivatives, patented by Pharmaceutical Institute are highly effective G2/M blockers, and show ability to disrupt mitotic spindles. The aim of a study is to test radiosensitizing properties of new genistein derivatives in prostate cancer cell lines. Project is held in cooperation with the Pharmaceutical Institute, Warsaw. [A. Rusin, arusin@io.gliwice.pl; Z. Krawczyk, krawczyk@io.gliwice.pl]

Gene expression profile of melanoma cells under hypoxic conditions. Hypoxia is an important feature of tumor microenvironment, exerting far-reaching effects on cells and contributing to cancer progression. Our high-density oligonucleotide microarrays based analysis performed on of B16 (F10) murine melanoma cells led to identification of several classes of genes differentially regulated by hypoxia. Currently, we work on validation of the gene signature of hypoxia *in vivo* (mouse model), as well as in human melanoma cell lines. In the near future we will extend microarray analysis to the human tumor cell lines (ovarian, prostate and breast cancer) grown in hypoxia. [M. Olbryt, molbryt@io.gliwice.pl]

The molecular mechanisms of a cell type-specific function of Heat Shock Transcription Factor 1 (HSF1). HSF1 is activated under stress conditions. In the majority of somatic cells activation of HSF1 leads to synthesis of heat shock proteins, which is a part of cytoprotective response. Our results indicate that in differentiating male germ cells HSF1 induces receptor-mediated and mitochondria-mediated apoptosis that is not prevented by HSP70i. The main aim of our work is to identify genes that are differentially expressed in somatic *versus* spermatogenic cells upon activation of HSF1. [Contact: Wieslawa Widlak, wwidlak@io.gliwice.pl]

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Selected Papers:

1. Raimondi S, Boffetta P, Anttila S, Bröckmoller J, Butkiewicz D, Cascorbi I, Clapper M L, Dragani T A, Garte S, Gsur A, Haidinger G, Hirvonen A, Ingelman-Sundberg M, Kalina I, Lan Q, Leoni V P, Marchand L L, London S J, Neri M, Povey A C, Rannug A, Reszka E, Ryberg D, Risch A, Romkes M, Ruano-Ravina A, Schoket B, Spinola M, Sugimura H, Wu X, Taioli E (2005): ***Metabolic gene polymorphisms and lung cancer risk in non-smokers, An update of the GSEC study.***
   BACKGROUND: Since genetic factors may play an important role in lung cancer development at low dose carcinogen exposure, non-smokers are a good model to study genetic susceptibility and its interaction with environmental factors. MATERIALS AND METHODS: We evaluated the role of the metabolic gene polymorphisms CYP1A1MspI, CYP1A1Ile462Val, GSTM1, and GSTT1 in non-smoker lung cancer patients from the International Collaborative Study on Genetic Susceptibility to Environmental Carcinogens (GSEC). Non-smokers (defined as subjects who never smoked on a regular basis) were selected from the GSEC database. We pooled the raw data from 21 case-control studies for a total of 2764 Caucasians (555 cases and 2209 controls) and 383 Asians (113 cases and 270 controls). Tests of heterogeneity and of inclusion bias were performed. RESULTS: A significant association between lung cancer and CYP1A1Ile462Val polymorphism was observed in Caucasians (adjusted OR=2.04, 95% CI 1.17-3.54). GSTT1 deletion seems to be a risk factor for lung cancer in Caucasian non smokers only when the analysis was restricted to studies including healthy controls (adjusted OR=1.66, 95% CI 1.12-2.46). A protective effect on lung cancer was observed with the combination of CYP1A1 wild type, GSTM1 null, and GSTT1 non-null genotypes. None of the analysed polymorphisms were associated with lung cancer in Asian non-smokers. DISCUSSION: Our analysis confirms previous findings that CYP1A1Ile462Val polymorphism may play a role in lung carcinogenesis in Caucasian non-smokers. *Mutat Res* **592**: 45-57

2. Sengupta S, Shimamoto A, Koshiji M, Pedeux R, Rusin M, Spillare A E, Shen J C, Huang L E, Lindor N M, Furuichi Y, Harris C C (2005) ***Tumor suppressor p53 represses transcription of RECQ4 helicase.***
   RECQ4 is a member of the RecQ helicase family, which has been implicated in the regulation of DNA replication, recombination and repair. p53 modulates the functions of RecQ helicases including BLM and WRN. In this study, we demonstrate that p53 can regulate the transcription of RECQ4. Using nontransformed, immortalized normal human fibroblasts, we show that p53-dependent downregulation of RECQ4 expression occurred in G1-arrested cells, both in the absence or presence of exogenous DNA damage. Wild-type p53 (but not the tumor-derived mutant forms) repressed RECQ4 promoter activity. The camptothecin or etoposide-dependent p53-mediated repression was attenuated by trichostatin A (TSA), an inhibitor of histone deacetylases (HDACs). Repression of the RECQ4 promoter was accompanied with an increased accumulation of HDAC1, and the loss of SP1 and p53 binding to the promoter. The simultaneous formation of a camptothecin-dependent p53-SP1 complex indicated its occurrence outside of the RECQ4 promoter. These data suggest that p53-mediated repression of RECQ4 transcription during DNA damage results from the modulation of the promoter occupancy of transcription activators and repressors. *Oncogene* **24**: 1738-1748

3. Dudaladava V, Jarząb M, Stobiecka E, Chmielik E, Simek K, Huzarski T, Lubiński J, amuła J, Pękala W, Grzybowska E, Lisowska K (2006): ***Gene expression profiling in hereditary, BRCA1-linked breast cancer: preliminary report.*** *Hereditary Cancer in Clinical Practice* **4**: 28-38

4. Kramer-Marek G, Serpa C, Szurko A, Wideł M, Sochanik A, Śnietura M, Kus P, Nunes R M D, Arnaut L G, Ratuszna A (2006): ***Spectroscopic properties and photodynamic effects of new lipophilic porphyrin derivatives: Efficacy, localisation and cell death pathways.***
   Photodynamic therapy (PDT) and photodynamic diagnostics (PDD) of cancer are based on the use of non-toxic dyes (photosensitisers) in combination with harmless visible light. This paper reports physicochemical properties, cell uptake, localisation as well as photodynamic efficiency of two novel lipophilic porphyrin derivatives, suitable for use as PDT sensitisers. Both compounds are characterised by high quantum yield of singlet oxygen generation which was measured by time-resolved phosphorescence.

Photodynamic in vitro studies were conducted on three cancer cell lines. Results of cell survival tests showed negligible dark cytotoxicity but high phototoxicity. The results also indicate that cell death is dependent on energy dose and time following light exposure. Using confocal laser scanning microscopy both compounds were found to localise in the cytoplasm around the nucleus of the tumour cells. The mode of cell death was evaluated based on the morphological changes after differential staining. In summary, good photostability, high quantum yield of singlet oxygen and biological effectiveness indicate that the examined lipophilic porphyrin derivatives offer quite interesting prospects of photodynamic therapy application. *J Photochem Photobiol B* **84**: 1-14

5. Małusecka E, Zborek A, Krzyżowska-Gruca S, Krawczyk Z (2006): ***Immunohistochemical detection of the inducible heat shock protein Hsp70. A methodological study.***
Stress-inducible Hsp70i and constitutively expressed Hsc70 are highly related heat shock proteins. Aberrant expression levels and intracellular localization of these proteins has been suggested as a potential marker in certain tumors. The aim of our study was to work out a reliable, immunohistochemical detection of the stress-inducible Hsp70i protein and enabling discrimination between Hsp70i and Hsc70 proteins in paraffin-embedded human tissues. We tested the effect of several fixative procedures and antigen retrieval on the effectiveness of the Hsp70i detection in murine cells cultured in vitro and in liver of rats subjected to heat shock. For cells grown in vitro, specific Hsp70i immunoreactivity was obtained with all fixatives used. However, samples fixed in 10% formalin and 4% paraformaldehyde required antigen retrieval. In liver tissue embedded in paraffin, regardless the fixative used, positive Hsp70i staining could be visible only if antigen retrieval was applied. We applied this procedure for detection of Hsp70i in routine sections of breast and lung cancers fixed with 10% formalin and found that the application of thermal antigen retrieval significantly enhanced the SPA810 immunoreactivity and reduced background staining. This procedure enabled also the differential detection of Hsp70i and Hsc70 in routine histopathological preparations. *J Histochem Cytochem* **54**: 183-190

6. Olbryt M, Jarząb M, Jazowiecka-Rakus J, Simek K, Szala S, Sochanik A (2006): ***Gene expression profile of B 16(F10) murine melanoma cells exposed to hypoxic conditions in vitro.***
Hypoxia is an important feature of tumor microenvironment, exerting far-reaching effects on cells and contributing to cancer progression. Previous studies have established substantial differences in hypoxia response between various cell lines. Investigating this phenomenon in melanoma cells contributes to a better understanding of cell lineage-specific hypoxia response and could point out novel hypoxia-regulated genes. We investigated transcriptional activity of B 16(F10) murine melanoma cells cultured for 24 h under hypoxic (nominal 1% O2, 15 samples including controls) and hypoxia-mimicking conditions (cobalt chloride, 100 or 200 microM, 6 samples including controls). Gene expression profiles were analyzed using MG-U74Av2 oligonucleotide microarrays. Data analysis revealed 2541 probesets (FDR <5%) for 1% oxygen experiment and 364 probesets (FDR <5%) for cobalt chloride, which showed differences in expression levels. Analysis of hypoxia-regulated genes (true hypoxia, 1% O2) by stringent Family-Wise Error Rate estimation indicated 454 significantly changed transcripts ($p < 0.05$). The most upregulated genes were Lgals3, Selenbpl, Nppb (more than ten-fold increase). We observed significant differences in expression levels of genes regulating glycolysis (Pfkp, Hk2, Aldo3, Eno2), apoptosis (Bnip3, Bnip31, Cdknla), transcription (Bhlhb2, Sap30, Atf3, Mxil), angiogenesis (Vegfa, Adm, Anxa2, Ctgf), adhesion (Pkp2, Itga4, Mcam), migration (Cnn2, Tmsb4x), and other processes. Both true hypoxia and hypoxia mimicry induced HIF-1-regulated genes. However, unsupervised analysis (Singular Value Decomposition) revealed distinct differences in gene expression between these two experimental conditions. Contrary to hypoxia, cobalt chloride caused suppression of gene expression rather than stimulation, especially concerning transcripts related to proliferation, immune response, DNA repair, and melanin biosynthesis. *Gene Expr* **13**: 191-203

7. Rupik W, Stawierej A, Stolarczyk I, Widłak W (2006): ***Promoter of the heat shock testis-specific Hsp70.2/Hst70 gene is active in nervous system during embryonic development of mice.***
The Hsp70.2/Hst70 gene is a unique member of the 70 kDa heat shock proteins multigene family whose activity is regulated developmentally; in adult mice and rats its expression is restricted mostly to meiotic and postmeiotic male germ cells. In aim to analyze activity of the Hsp70.2/Hst70 promoter in developing embryos we have constructed transgenic mice expressing EGFP reporter gene under control of the rat Hst70 promoter. The appearance of EGFP fluorescence coincides with series of major developmental events, such as extra-embryonic membranes formation, axial rotation, formation of neural tube and the

primordium of central nervous system, formation of differentiated somites, extensive remodeling of the heart, development of fingers and toes, and sensory organs formation. Activity of the Hst70 promoter localizes mostly inside nervous system indicating the role of Hsp70.2/Hst70 gene in development of this system. *Anat Embryol* **211**: 631-638

8. Vydra N, Małusecka E, Jarząb M, Lisowska K, Głowala-Kosińska M, Benedyk K, Widłak P, Krawczyk Z, Widłak W (2006) ***Spermatocyte-specific expression of constitutively active heat shock factor 1 induces HSP70i-resistant apoptosis in male germ cells.***
   Spermatocytes, the most sensitive male germ cells to heat-induced apoptosis, do not respond to hyperthermia by inducing heat shock proteins (HSPs), including HSP70i, which has been previously shown to confer resistance to apoptosis in somatic cells. To dissect the mechanism of heat-induced apoptosis and to determine if we could protect spermatocytes by expressing HSP70i, we engineered transgenic mice that express in spermatocytes constitutively active heat shock transcription factor (HSF)1. Such HSF1 expression did not lead to transcription of inducible Hsp70 genes, but instead induced caspase-dependent apoptosis that mimicked heat shock-induced death of spermatogenic cells. Both mitochondria-dependent and death receptor-dependent pathways appear to be involved in such HSF1-induced apoptosis: the levels of Bcl-2 family proteins became increased, p53 protein accumulated and expression levels of caspase-8 and death-receptor-interacting proteins (including Fas-associated death domain protein and TNF receptor associated death domain protein) became elevated. Surprisingly, the constitutive spermatocyte-specific expression of HSP70i in double-transgenic males did not protect against such HSF1-induced apoptosis. *Cell Death Differ* **13**: 212-222

9. Zborek A, Małusecka E, Rusin A, Krzyżowska-Gruca S, Krawczyk Z (2006): ***Influx of macrophages into livers of rats treated with hepatotoxicants (thioacetamide, allyl alcohol, D-galactosamine) induces expression of HSP25.***
   Treatment of rats with a single dose of thioacetamide (TAA) provokes centrilobular inflammation and a significant expression of heat shock protein HSP25 in hepatocytes surrounding the area of inflammation. The HSP25 accumulation in hepatocytes adjacent to inflammatory regions was confirmed by identification of positive hepatocytes concentrated at periportal areas after treatment of rats with allyl alcohol (AA) or distributed diffusely throughout liver lobule after treatment with D-galactosamine (D-gal). In our model of TAA-treated rats the use of the anti-inflammatory drug-indomethacin, and the redox-regulating drug-N-acetylcysteine (NAC), significantly attenuated TAA-induced HSP25 expression and evoked morphological changes of recruited ED1+ macrophages. Treatment of rats with gadolinium chloride (GdCl(3)) decreased considerably the number of Kupffer cells (ED2+ macrophages) without affecting significantly the number and morphology of ED1+ macrophages as well as the expression pattern of TAA-induced HSP25. Our data shows for the first time that ED1+ macrophages recruited into the liver by treatment with TAA play a significant role in HSP25 induction in hepatocytes. *J Mol Histol* **37**: 381-389

Department of Molecular Biology

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

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

1. Novel antivascular strategies. Novel antitumor strategies: combination of antivascular targeting agents and anticancer drugs.

2. Two-domain antivascular drugs. Novel drug carriers.

3. Epidemiology of breast cancer; hereditary predisposition to breast and ovarian cancers; identification of new genes modifying predisposition to breast cancer.

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Selected Papers:

1. Mitrus I, Missol-Kolka E, Płucienniczak A, Szala S (2005): ***Tumour therapy with genes encoding apoptin and E4orf4.***
   The aim of our study was to investigate whether apoptin and e4orf4 pro-apoptotic genes, transferred by means of electroporation, were suitable for gene therapy of tumours. The two genes were chosen for our study because the proteins they encode induce apoptosis in transformed cells only. The apoptin gene was synthesised based on a published nucleotide sequence. MTT and TUNEL tests confirmed that both the synthesised apoptin gene and the e4orf4 gene indeed induced apoptosis in COS-7, Renca and B16(F10) cell lines. Therapeutic DNA was then administered via electroporation directly into murine B16(F10) tumours. Distinct tumour growth inhibition was seen only during the treatment. The cessation of therapy caused tumour re-growth. Obviously, the efficiency of gene transfer using electroporation is low and did not induce a permanent therapeutic effect. *Anticancer Res* **25**: 1087-1090

2. Smagur A, Szary J, Szala S (2005): ***Recombinant angioarrestin secreted from mouse melanoma cells inhibits growth of primary tumours.***
   Angioarrestin is a recently described anti-angiogenic protein whose expression is down-regulated in solid tumours of various origins. It has a sequence identical to angiopoietin related protein-1. In this study we investigated anti-tumour properties of angioarrestin in B16 (F10) melanoma tumour model. We constructed an expression vector encoding human angioarrestin under the control of EF-1alpha promoter. This vector was transferred to B16 (F10) cells and recombinant angioarrestin secreted from the transfected cells was tested for anti-angiogenic activity using endothelial cell proliferation assay. Finally, mice were injected subcutaneously with cells that had been transfected with either angioarrestin-encoding vector or empty vector and tumor growth was compared. The obtained recombinant angioarrestin inhibited proliferation of bovine aortic endothelial cells. Tumours derived from an angioarrestin-secreting B16 (F10) cell clone grew in vivo more slowly than tumours derived from a cell clone transfected with empty vector. These data show, to our knowledge for the first time, that angioarrestin can inhibit primary melanoma tumour growth. *Acta Biochim Polon*. **52**: 875-879

3. Smolarczyk R, Cichoń T, Sochanik A, Szala S (2005): ***Negligible induction of IFN-g, IL-12, TNF-a by DNA-PEI 750kDa/albuminum complexes.***
   A 750 kDa polyethylenimine (PEI 750 kDa) combined with albumin has been found to mediate in vivo a highly efficient transfection of small amounts of plasmid DNA. Using this exceptional carrier system we evaluated the inflammatory responses triggered by CpG sequences found in plasmid DNA. Using as little as 1 mug DNA transferred in vivo caused an almost negligible response from pro-inflammatory cytokines (IFN-gamma, IL-12 and TNF-alpha), as assessed in serum with a commercially available kit. Administering 750 kDa PEI/albumin/plasmid DNA complexes every three days assured a high and prolonged in vivo expression of a reporter protein. A further increase in the level of such protein was obtained by administering the investigated complexes concurrently with dexamethasone. High gene transfer capability and a relatively low pro-inflammatory response of 750 kDa PEI/albumin/DNA complexes can be exploited for recurrent gene transfer into lungs to treat (via inhalation or instillation) cancer or genetic disorders such as cystic fibrosis. *Cytokine* **29**: 283-287

4. Wagner K, Hemminki K, Israelson E, Grzybowska E, Soderberg M, Pamuła J, Pękala W, Zientek H, Mielżyńska D, Siwińska E, Forsti A (2005): ***Polymorphisms in the IGF-1 and IGFBP3 promoter and the risk of breast cancer.***
   Binding of IGF-1 to the type I IGF receptor starts a signalling cascade that plays an important role in regulating cell proliferation, differentiation and apoptosis. The interaction between the IGF-1 and its receptor is mainly regulated by a binding protein, IGFBP 3. We studied a CA repeat polymorphism 969 bp upstream of the transcription start site in the IGF-1 gene and an A-202 C polymorphism in the IGFBP 3 gene and tested their association with breast cancer risk using four case-control series with a total of 787 cases and 900 controls. We did not find any association between the breast cancer risk and the IGF-1 repeat length (19 versus non-19) or the IGFBP 3 A-202 C polymorphism in the postmenopausal breast cancer series or in women diagnosed for breast cancer under the age of 50. In the familial breast cancer series we observed a non-significantly increased odds-ratio (OR) in homozygotes for the non-19 alleles of the IGF-1 gene (OR 1.51, 95% CI 0.96-2.39, p=0.07). Similarly, in the familial breast cancer series we detected an increased frequency of the IGFBP 3 -202 C allele carriers (OR 1.50, 95% CI 1.05--2.14, p=0.03). The

association was stronger in individuals homozygous for these alleles (OR 3.76, 95% CI 1.44-v-9.81, p=0.006). Thus, the polymorphisms in the IGF-1 and IGFBP 3 genes associated with an increased risk of breast cancer in familial cases carrying the variant alleles. *Breast Cancer Res Treat* **92**: 133-140

5. Wagner K, Hemminki K, Israelsson E, Grzybowska E, Klaes R, Chen B, Butkiewicz D, Pamula J, Pekala W, Försti A (2005): ***Association of polymorphisms and haplotypes in the human growth hormone 1 (GH1) gene with breast cancer.***
The growth hormone 1 (GH1)/insulin-like growth factor I (IGF-I) axis plays an important role in the development of breast cancer. By binding to its receptor, GH1 stimulates the production of IGF-I and its binding protein IGFBP3, resulting in the regulation of cell proliferation, differentiation and apoptosis. The GH1 gene expression is regulated by a highly polymorphic proximal promoter and a distal locus control region (LCR) 14.5 kb upstream of the gene. We investigated the effect of single nucleotide polymorphisms (SNPs) in the LCR and in the promoter region and an intron 4 SNP (IVS4+90 T/A) on breast cancer risk in a large cohort of Polish and German familial breast cancer cases and controls. SNPs in the LCR did not show an influence on breast cancer risk, either alone or in haplotypes. Three SNPs in the promoter region (G-340T, A-68G/C and A-63T/C) showed an increased and four SNPs (A-137G, G-119T, G-93delG and T-4G) a decreased allele frequency in the cases compared with the controls. Two of the SNPs (A-137G and G-93delG) lead to a decreased breast cancer risk among the minor allele carriers in the joint analysis of the two populations (odds ratio (OR) 0.62, 95% confidence interval (95% CI) 0.44-0.89, P = 0.01 and OR 0.65, 95% CI 0.47-0.90, P = 0.01, respectively). Haplotype analysis with these seven promoter SNPs revealed a protective association (OR 0.61, 95% CI 0.37-1.00, P = 0.04) for the haplotype GAGdAAT, containing the G-93delG variant allele, which in the single analysis already showed a protective effect. The effect was marginally stronger in combination with the LCR GC haplotype (OR 0.49, 95% CI 0.23-1.01, P = 0.04). *Endocrine-Related Cancer* **12**: 917-928

6. Wirtenderger M, Hemminki K, Forsti A, Klaes R, Schmutzler R K, Grzybowska E, Bermejo J L, Wappenschmidt B, Bugert P, Butkiewicz D, Pamuła J, Pękala W, Zientek H, Bertram C R, Burwinkel B (2005): ***c-MYC Asn11Ser is associated with increased risk for familial breast cancer.***
c-MYC is a multifaceted protein that regulates cell proliferation, differentiation and apoptosis. Its crucial role in diverse cancers has been demonstrated in several studies. Here, we analysed the influence of the rare c-MYC Asn11Ser polymorphism on familial breast cancer risk by performing a case-control study with a Polish (cases n = 349; controls n = 441) and a German (cases n = 356; controls n = 655) study population. All cases have been tested negative for mutations in the BRCA1 and BRCA2 genes. A joint analysis of the Polish and the German study population revealed a 54% increased risk for breast cancer associated with the heterozygous Asn11Ser variant (OR = 1.54, 95% CI 1.05-2.26, p = 0.028). The breast cancer risk associated with this genotype increases above the age of 50 years (OR = 2.24, 95% CI 1.20-4.21, p = 0.012). The wild-type amino acid Asn of this polymorphism is located in the N-terminal MYC transactivation domain and is highly conserved not only among most diverse species but also in the N-MYC homologue. Due to the pivotal role of c-MYC in diverse tumours, this variant might affect the genetic susceptibility of other cancers as well. *Int. J. Cancer* **117**: 638-642

7. Cichoń T, Smolarczyk R, Sochanik A, Szala S (2006): ***Plasmid DNA-induced cytokines together with cyclophosphamide decrease size and number of melanoma lung metastases.***
The aim of this study was to investigate whether the local induction of pro-inflammatory cytokines in mouse lungs would increase the therapeutic effect of cyclophosphamide (CTX) used to treat experimental B16(F10) melanoma lung metastases. CTX shows antiangiogenic properties and inhibits the growth of metastases, albeit without numerical reduction. To destroy small metastases remaining after CTX treatment, pro-inflammatory cytokines were induced by systemically administering plasmid DNA-PEl polyplexes. The CpG sequences present in plasmid DNA are immunostimulatory, i.e. they induce pro-inflammatory cytokines, such as IL-12, TNF-alpha, IFN-gamma and IFN-alpha. The latter has great therapeutic potential as it activates NK cells directly involved in eliminating metastatic foci. Our data indicated, for the first time, that combining cyclophosphamide delivery and local induction of pro-inflammatory cytokines in the lungs with plasmid DNA resulted in reduction in the size of malignant melanoma metastases and their number in mouse lungs. Both effects appeared to contribute to a significant extension of survival. *Anticancer Res* **26**: 2033-2036

8. Jazowiecka-Rakus J, Jarosz M, Szala S (2006): ***Combination of vasostatin gene therapy with cyclophosphamide inhibits growth of B16(F10) melanoma tumors.***
Angiogenesis, i.e. formation of new blood vessels out of pre-existing capillaries, is essential to the development of tumour vasculature. The discovery of specific antiangiogenic inhibitors has important therapeutic implications for the development of novel cancer treatments. Vasostatin, the N-terminal domain of calreticulin, is a potent endogenous inhibitor of angiogenesis and tumour growth. In our study, using B16(F10) murine melanoma model and electroporation we attempted intramuscular transfer of human vasostatin gene. The gene therapy was combined with antiangiogenic drug dosing schedule of a known chemotherapeutic (cyclophosphamide). The combination of vasostatin gene therapy and cyclophosphamide administration improved therapeutic effects in melanoma tumours. We observed both significant inhibition of tumour growth and extended survival of treated mice. To our knowledge, this is one of the first reports showing antitumour efficacy of electroporation-mediated vasostatin gene therapy combined with antiangiogenic chemotherapy. *Acta Biochim Polon* **53**: 199-202

9. Lubinski J, Gorski B, Huzarski T, Byrski T, Gronwald J, Serrano-Fernandez P,Domagala W, Chosia M, Ucinski M, Grzybowska E, Lange D, Maka B, Mackiewicz A,Karczewska A, Breborowicz J, Lamperska K, Stawicka M, Gozdecka-Grodecka S, i inni (2006): ***BRCA1-positive breast cancers in young women from Poland.***
We identified 4316 unselected incident cases of early-onset breast cancers (<51 ears of age at diagnosis) in 18 Polish hospitals between 1996 and 2003. We were able to obtain a blood sample for DNA analysis from 3472 of these (80.4%). All cases were tested for the presence of three founder mutations in BRCA1. The proportion of cases with a BRCA1 mutation was 5.7%. The hereditary proportions were higher than this for women with breast cancer diagnosed before age 40 (9%), for women with cancer of medullary or atypical medullary histology (28%), for those with bilateral cancer (29%) or with a family history of breast or ovarian cancer (13%). It is reasonable to offer genetic testing to women with early-onset breast cancer in Poland. *Breast Cancer Res Treat* **99**: 71-6

10. Mitrus I, Delić K, Wróbel N, Missol-Kolka E, Szala S (2006): ***Combination of IL-12 gene therapy and CTX chemotherapy inhibits growth of primary B16(F10) melanoma tumors in mice.***
We investigated suppression of murine B16(F10) melanoma tumor growth following a therapy which involved concomitant administration of cyclophosphamide and plasmid DNA bearing interleukin-12 gene. Since both therapeutic factors display antiangiogenic capabilities, we assumed that their use in blocking the formation of new blood vessels would result in augmented inhibition of tumor growth. This combined therapy regimen indeed resulted in a considerable suppression of tumor growth. We observed a statistically significant extension of treated animals' lifespan. Interestingly, the therapeutic effect was also obtained using a plasmid without an interleukin gene insert. This observation suggests that plasmid DNA, which has been widely applied for treating neoplastic tumors, contains element(s) that elicit immune response in mice. *Acta Biochim Polon* **53**: 357-360

11. Smolarczyk R, Cichon T, Graja K, Hucz J, Sochanik A, Szala S (2006): ***Antitumor effects of RGD-4C-GG-D(KLAKLAK)2 peptide in mouse B16(F10) melanoma model.***
Vasculature targeting agents have been tested as cancer therapeutics for the past few years. Such therapy could be accomplished using, for example, bifunctional (two-domain) peptides. RGD-4C-GG-(D)(KLAKLAK)(2), a peptide designed by Ellerby and coworkers (1999) (full sequence: ACDCRGDCFCGGKLAKLAKKLAKLAK), binds selectively to alpha(V)beta(3) integrin receptors expressed in tumor neovasculature and, after internalization, effectively induces apoptosis of endothelial cells. The aim of this study was to examine if RGD-4C-GG-(D)(KLAKLAK)(2) would efficiently target cells, among them B16(F10), that overexpress alpha(V)beta(3) receptors, and whether it would be suitable for therapeutic treatment of primary B16(F10) murine melanoma tumors. Thus, the peptide would target two distinct tumor compartments: that formed by endothelium of blood vessels and that made up of neoplastic cells. The therapeutic peptide was recognized and did induce apoptosis in B16(F10) cell line. Tumor growth inhibition was observed following direct intratumoral administration. However, cessation of peptide administration led to rapid tumor growth and death of the animals. *Acta Biochim Polon* **53**: 801-805

12. Wagner K, Hemminki K, Grzybowska E, Bermejo J L, Butkiewicz D, Pamuła J, Pękala W, Försti A (2006): ***Polymorphisms in the growth hormone receptor: A case-control study in breast cancer.***
The human growth hormone receptor (GHR) mediates the effects of growth hormone (GH1), starting a signalling cascade that is involved in the regulation of proliferation, differentiation and apoptosis. Recently, an isoform of the GHR gene lacking exon 3 (GHRd3) was associated with accelerated responsiveness to growth hormone. In this study, we investigated the association of the GHRd3 polymorphism with breast cancer risk and performed a haplotype analysis with 3 additional single nucleotide polymorphisms (SNPs) (Gly186Gly, Cys440Phe and Ile544Leu) in the GHR coding region in a Polish cohort. We did not observe any effect of the 4 polymorphisms on breast cancer risk. Neither did the 3 most common haplotypes influence breast cancer risk. However, a rare haplotype (dGGC), containing the GHRd3 allele, was associated with a decreased breast cancer risk (OR 0.30, 95% CI 0.11-0.80). *Int J Cancer* **118**: 2903-2906

13. Wagner K, Hemminki K, Grzybowska E, Klaes R, Burwinkel B, Bugert P, Schmutzler R K, Wappenschmidt B, Butkiewicz D, Pamuła J, Pękala W, Förstil A (2006): ***Polymorphisms in genes involved in GH1 release and their association with breast cancer risk.***
The regulation of growth hormone 1 (GH1) and insulin-like-growth factor-1 (IGF-1) release is under the influence of three pituitary hormones [growth hormone releasing hormone (GHRH), ghrelin (GHRL) and somatostatin (SST)], which act in an autocrine/paracrine fashion in the breast. By binding to their respective receptors, they control cell proliferation, differentiation and apoptosis in a GH1/IGF-1-dependent manner. We investigated single nucleotide polymorphisms (SNPs) in the GHRH, GHRHR, GHRL, GHSR, SST and SSTR2 gene regions in a Polish and a German cohort of 798 breast cancer cases and 1011 controls. Our study revealed an association of a novel TC repeat polymorphism in the SST promoter with a decreased breast cancer risk in the Polish study population [odds ratio (OR), 0.65; 95% confidence interval (CI), 0.44-0.96]. The closely linked SNP IVS1 A+46G showed the same trend. For both polymorphisms the association was stronger in women above the age of 50 (OR, 0.33; 95% CI, 0.14-0.76 and OR, 0.39; 95% CI, 0.18-0.87, respectively). The protective effect of these polymorphisms was confirmed in a haplotype analysis among women above 50 years of age and carrying the two variant alleles (OR, 0.37; 95% CI, 0.17-0.80). In the independent German population, we observed slightly decreased ORs among women above the age of 50 years. In the SSTR2 gene, carriers of the promoter 21/21 TG repeat genotype were at a decreased breast cancer risk (OR, 0.62; 95% CI, 0.41-0.94) compared to carriers of the other genotypes in the Polish population. Furthermore, we identified a protective effect of the GHRHR C-261T SNP in both populations (joint analysis CT+TT versus CC: OR, 0.80; 95% CI, 0.65-0.99). This effect was carried by a haplotype containing the protective allele. Thus, our study concludes a possible protective influence of distinct polymorphisms in genes involved in GH1 release on breast cancer risk. *Carcinogenesis* **27**: 1867-1875

14. Was H, Cichon T, Smolarczyk R, Rudnicka D, Stopa M, Chevalier C, Legger JJ, Lackowska B, Grochot A, Bojkowska K, Rtajska A, Kieda C, Szala S, Dulak J, Jozkowicz A (2006): ***Overexpression of heme oxygenase-1 in murine melanoma: increased proliferation and viability of tumor cells, decreased survival of mice.***
Heme oxygenase-1 (HO-1), a cytoprotective enzyme, can be induced in tumors in response to anti-cancer therapies. We investigated the role of HO-1 in B16(F10), S91, and Sk-mel188 melanoma cells. Overexpression of HO-1 after transduction with adenoviral vectors increased cell proliferation, resistance to oxidative stress generated by H2O2, and angiogenic potential as determined by induction of endothelial cell divisions. Likewise, cells stably transfected with HO-1 cDNA (B16-HO-1) showed higher proliferation, stress resistance, and angiogenic activity than the wild-type line (B16-WT). HO-1 overexpression in tumors significantly shortened survival of mice after subcutaneous injection of cancer cells (38 and 22 days for B16-WT and B16-HO-1, respectively; P=0.017). This also resulted in development of more packed tumors, with more melanoma cells, and reduced inflammatory edemas. Mice injected with B16-HO-1 had lower levels of tumor necrosis factor and higher serum concentrations of its soluble receptor tumor necrosis factor-RI, whereas tumors overexpressing HO-1 displayed augmented vascularization and stronger production of vascular endothelial growth factor. Finally, B16-HO-1 cells injected intravenously formed more metastases in lungs. Thus, HO-1 overexpression increased viability, proliferation, and angiogenic potential of melanoma cells, augmented metastasis, and decreased survival of tumor-bearing mice, suggesting that induction of HO-1 may be detrimental in anti-cancer therapy of melanoma. *Am J Pathol*. 69: 2181-98

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.

Main topics of interest:

- DNA damage and repair, studied on normal and tumor cells:
  - the characterization of proteins which recognize DNA damage and participate in its repair;
  - the influence of antioxidants on DNA damage induced by radiation;
- The individual variability of radiosensitivity of cancer patients and healthy subjects, tested in vitro by genetic, cytogenetic and cellular tests.
- The comparative assessment of the effectiveness of conventional, accelerated or continuous schemes of radiation dose fractionation:
- The identification of genes and proteins which are involved in cellular responses to radiation and modulate the radiation sensitivity of normal and malignant cells.
- The regulation of molecular processes involved in terminal stages of apoptosis: DNA fragmentation and chromatin condensation.
- Mathematical modelling of regulatory circuits of signaling pathways involved in cancer cell biology

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Selected Papers:

1. Kalinowska M, Garncarz W, Pietrowska M, Garrard W T, Widłak P (2005): ***Regulation of the human apoptotic DNase/RNase EndoG; involvement of Hsp70 and ATP.***
   Endonuclease G (EndoG) is a mitochondrial enzyme that becomes an apoptotic nuclease when released from the mitochondrial intermembrane space. EndoG will digest either DNA or RNA, but at physiological ionic strength, RNA is a much more favorable substrate as compared to chromatin. This indicates that EndoG's major in vivo function(s) may be: (i) an apoptotic RNase, and/or (ii) an apoptotic DNase in the presence of additional co-activators. In the present study we have searched for factors that modulate the activity of human EndoG on DNA substrates. We demonstrate that EndoG forms complexes with AIF and FEN-1 but not with PCNA. Interestingly, heat shock proteins 70 interact with EndoG and are involved in the regulation of its activity. Purified Hsp70 prevented stimulation of EndoG DNase activity by other nuclear factors in the ATP-dependent manner. *Apoptosis* 10: 821-830

2. Pietrowska M, Widłak P (2005): ***Characterization of a novel protein that specifically binds to DNA modified by N-acetoxy-acetylaminofluorene and cis-diammine-dichloro-platinum.***
   Proteins recognizing DNA damaged by the chemical carcinogen N-acetoxy-acetylaminofluorene (AAAF) were analyzed in nuclear extracts from rat tissues, using a 36 bp oligonucleotide as a substrate and electrophoretic mobility shift and Southwestern blot assays. One major damage-recognizing protein was detected, whose amount was estimated as at least 10(5) copies per cell. Levels of this protein were similar in extracts from brain, kidney and liver, but much lower in extracts from testis. The affinity of the detected protein for DNA damaged by AAAF was about 70-fold higher than for undamaged DNA. DNA damaged by cis-diamminedichloroplatinum (cis-DDP), benzo(a)pyrene diolepoxide (BPDE) or UV-radiation also bound this protein with an increased affinity, the former more strongly and the latter two more weakly as compared to AAAF-damaged DNA. The detected AAAF/DDP-damaged-DNA-binding (AAAF/DDP-DDB) protein had a molecular mass of about 25 kDa and was distinct from histone H1 or HMGB proteins, which are known to have a high affinity for cis-DDP-damaged DNA. The level of this damage-recognizing protein was not affected in rats treated with the carcinogen 2-acetylaminofluorene. The activity of an AAAF/DDP-DDB protein could also be detected in extracts from mouse liver cells but not from the Hep2G human hepatocellular carcinoma. *Acta Biochim Polon* **52**: 867-874

3. Ryabokon N I, Goncharova R I, Duburs G, Rzeszowska-Wolny J (2005): ***A1,4-dihydro-pyridine derivative reduces DNA damage and stimulates DNA repair in human cells in vitro.***
   Compounds of the 1,4-dihydropyridine (1,4-DHP) series have been shown to reduce spontaneous, alkylation- and radiation-induced mutation rates in animal test systems. Here we report studies using AV-153, the 1,4-DHP derivative that showed the highest antimutagenic activity in those tests, to examine if it modulates DNA repair in human peripheral blood lymphocytes and in two human lymphoblastoid cell lines, Raji and HL-60. AV-153 caused a 50% inhibition of growth (IC50) of Raji and HL-60 cells at 14.9+/-1.2 and 10.3+/-0.8mM, respectively, but did not show a cytotoxic effect at concentrations <100 microM. Alkaline single-cell gel electrophoresis (comet) assays showed that AV-153 reduced the number of DNA strand breaks in untreated cells and also in cells exposed to 2 Gy of gamma-radiation, 100 microM ethylmethane sulfonate (EMS), or 100 microM H2O2. DNA damage was reduced by up to 87% at AV-153 concentrations between 1 and 10nM, and a positive dose-effect relationship was seen between 0.01 and 1 nM. Comparison of the kinetics of DNA strand-break rejoining in the presence and absence of AV-153 revealed a considerable influence on the rate of repair. In view of the resemblance of this compound's structure to that of dihydronicotinamide, a substrate for poly(ADP-rybose)polymerase, the modulation of DNA repair by AV-153 could involve an influence on poly(ADP)ribosylation. *Mutat Res.* **10**: 52-58

4. Rzeszowska-Wolny J, Polańska J, Pietrowska M, Palyvoda O, Jaworska, Butkiewicz D, Hancock R (2005)***: Influence of Polymorphisms in DNA Repair Genes XPD, XRCC1 and MGMT on DNA Damage Induced by Gamma Radiation and its Repair in Lymphocytes in vitro.***
   DNA single-strand breaks (SSBs) were quantified by single-cell gel electrophoresis and micronucleated and apoptotic cells were quantified by microscopic assays in peripheral blood lymphocytes after irradiation

on ice with 2 Gy of 60Co gamma radiation, and their association with polymorphisms of genes that encode proteins of different DNA repair pathways and influence cancer risk (XPD codon 312Asp --> Asn and 751Lys --> Gln, XRCC1 399Arg --> Gln, and MGMT 84Leu --> Phe) was studied. In unirradiated lymphocytes, SSBs were significantly more frequent in individuals older than the median age (52 years) ($P = 0.015$; $n = 81$), and the frequency of apoptotic or micronucleated cells was higher in individuals with alleles coding for Asn at XPD 312 or Gln at 751 ($P = 0.030$ or 0.023 ANOVA, respectively; $n = 54$). The only polymorphism associated with the background SSB level was MGMT 84Phe ($P = 0.04$, ANOVA; $n = 66$). After irradiation, SSB levels and repair parameters did not differ significantly with age or smoking habit. The SSB level varied more than twofold and the repair rate and level of unrepaired SSBs more than 10-fold between individuals. The presence of variant alleles coding for Asn at XPD 312 was associated with more radiation-induced SSBs ($P = 0.014$) and fewer unrepaired SSBs ($P = 0.008$), and the phenotype (> median induced SSBs/< median unrepaired SSBs) was seen in the majority of XPD 312Asn/Asn homozygotes; the odds ratio for variant homozygotes to show this phenotype was 5.2 (95% confidence interval 1.4-19.9). The hypothesis is discussed that XPD could participate in repair of ionizing radiation-induced DNA damage. While it cannot be excluded that the effects observed are due to cosegregating polymorphisms or that the responses of lymphocytes are not typical of other cell types, the results suggest that polymorphism of DNA repair genes, particularly XPD, is one factor implicated in the variability of responses to ionizing radiation between different individuals. *Radiation Res.* **164**: 132-140

5. Walichiewicz P, Przybyszewski WM, Snietura M, Lange D, Bkhiyan A, Widel M (2005): ***Protective effect of local temporary ischemia depends on applied dose of radiation.***
The aim of this study was to verify hypothesis that protective effect of local temporary ischemia depends on dose of radiation. 56 male WAG-strain rats were used. Total body irradiation with 3 x 3 and 3 x 5 Gy was performed. Local temporary ischemia was induced by clamping the tail base. The biochemical parameters were the thiobarbituric acid-reactive substances (TBA-RS). In bone marrow smears the polychromatic erythrocyte (PCE) numbers were counted and the numbers of micronucleated PCEs were analyzed. In small intestines the numbers of crypts were calculated. The levels of TBA-RS in the serum of the animals irradiated with a 3 x 3 Gy dose were significantly different ($P < 0.002$). Also in animals irradiated with a dose of 3 x 3 Gy the numbers of intestinal crypts were different ($P < 0.05$). In animals irradiated with dose 3 x 5 Gy, for analyzed parameters differences did not achieve statistical significance. Local temporary ischaemia provides general protection against radiation damage for lower dose. This protective effect disappeared after applications of a higher dose of radiation. *Cancer Lett* **10**: 113-138

6. Wideł M, Słowiński J, Mazurek U, Macyszyn G B, Latocha M, Ligus J G, Stomal M, Mrowka R (2005): ***Cell proliferative activity by histone H2B mRNA level correlates with cytogenetic damage induced by radiation in human glioblastoma cell lines.***
We studied the relationship between proliferative activity and radiation-induced DNA damage in human malignant gliomas in vitro. Nine human glioblastoma established cell lines were gamma-irradiated (60Co) over a dose range of 0-10 Gy. H2B and H4 histone mRNA level was assessed with quantitative RT-PCR technique (TaqMan) and histone labeling index (HLI) with in situ hybridization to define proliferation rate, while cytochalasin-block micronucleus assay was performed to measure cytogenetic damage. Micronucleus frequency correlated with H2B mRNA level (Spearman's R up to 0.82 at 8 Gy), HLI, nuclear division index (NDI) and percentage of binucleated cells (%BNC). There was a high correlation between H2B mRNA level and NDI ($R = 0.80$) as well as %BNC and HLI ($R = 0.72$). Histone H2B and H4 mRNA level (not significant), HLI, NDI, and %BNC (significant) were higher in cell lines sensitive to DNA damage. Proliferative activity correlates with radiation-induced DNA damage in human glioma cell lines. Histone H2B mRNA level and HLI may be a useful molecular predictor of the tumour response to radiation treatment in gliomas of the same histological grade, however the risk of potentially more rapid tumour-cell repopulation must be considered. Presumed protective activity of histones against radiation-induced DNA damage was not confirmed at the transcript level. *Journal of Neuro-Oncology* **71**: 237-243

7. Widlak P, Garrard WT (2005): ***Discovery, regulation, and action of the major apoptotic nucleases DFF40/CAD and endonuclease G.***
Toward the end of the 20th and beginning of the 21st centuries, clever in vitro biochemical complementation experiments and genetic screens from the laboratories of Xiaodong Wang, Shigekazu Nagata, and Ding Xue led to the discovery of two major apoptotic nucleases, termed DNA fragmentation factor (DFF) or caspase-activated DNase (CAD) and endonuclease G (Endo G). Both endonucleases attack chromatin to yield 3'-hydroxyl groups and 5'-phosphate residues, first at the level of 50-300 kb cleavage

products and next at the level of internucleosomal DNA fragmentation, but these nucleases possess completely different cellular locations in normal cells and are regulated in vastly different ways. In non-apoptotic cells, DFF exists in the nucleus as a heterodimer, composed of a 45 kD chaperone and inhibitor subunit (DFF45) and a 40 kD latent nuclease subunit (DFF40/CAD). Apoptotic activation of caspase-3 or -7 results in the cleavage of DFF45/ICAD and release of active DFF40/CAD nuclease. DFF40's nuclease activity is further activated by specific chromosomal proteins, such as histone H1, HMGB1/2, and topoisomerase II. Endo G resides in the mitochondrial intermembrane space in normal cells, and is released into the nucleus upon apoptotic disruption of mitochondrial membrane permeability in association with co-activators such as apoptosis-inducing factor (AIF). Understanding further regulatory check-points involved in safeguarding non-apoptotic cells against accidental activation of these nucleases remain as future challenges, as well as designing ways to selectively activate these nucleases in tumor cells. *J Cell Biochem* **15**: 1078-1087

8. Widłak P, Kalinowska M, Parseghian M H, Lu X, Hansen J C, Garrard W T (2005): ***The histone H1 C-terminal domain binds to the apoptotic nuclease, DNA Fragmentation Factor (DFF40/CAD) and stimulates DNA cleavage.***
The apoptotic nuclease, DNA fragmentation factor (DFF40/CAD), is primarily responsible for internucleosomal DNA cleavage during the terminal stages of programmed cell death. Previously, we demonstrated that histone H1 greatly stimulates naked DNA cleavage by this nuclease. Here, we investigate the mechanism of this stimulation with native and recombinant mouse and human histone H1 species. Using a series of truncation mutants of recombinant histone H1-0, we demonstrate that the H1 C-terminal domain (CTD) is responsible for activation of DFF40/CAD. We show further that the intact histone H1-0 CTD and certain synthetic CTD fragments bind to DFF40/CAD and confer upon it an increased ability to bind to DNA. Interestingly, we find that each of the six somatic cell histone H1 isoforms, whose CTDs differ significantly in primary sequence but not amino acid composition, equally activate DFF40/CAD. We conclude that the interactions identified here between the histone H1 CTD and DFF40/CAD target and activate linker DNA cleavage during the terminal stages of apoptosis. *Biochemistry* **44**: 7871-7878

9. Konopacka M, Rzeszowska-Wolny J (2006): ***The bystander effect-induced formation of micronucleated cells is inhibited by antioxidants, but the parallel induction of apoptosis and loss of viability are not affected.***
X-rays induce various DNA damages including strand breaks that lead to formation of micronuclei and chromosomal aberrations as well as increased number of apoptotic cells. Similar effects appear when non-irradiated cells are treated with medium collected from cultures of irradiated cells (irradiation conditioned medium - ICM). This phenomenon was termed "bystander effect". A number of studies suggest that bystander effect appears to be associated with up-regulation of oxidative metabolism. We thus compared the effects of antioxidant Vitamins C and E on the frequency of micronuclei and apoptotic cells in both directly irradiated cell cultures and in cultures exposed to ICM. Addition of Vitamins C or E (1-40 microg/ml) to culture medium after exposure to radiation or ICM reduced the frequency of micronuclei in a concentration-dependent manner. These vitamins had no effect on cell viability, clonogenic survival or the frequency of apoptotic cells under both conditions tested. These results show that the bystander effect causes micronucleation in addition to other known effects and suggest that the factors causing micronucleation by X-irradiation, oxidative DNA damage and incomplete repair, are regulated by apoptosis-independent pathways. *Mutation Res* **593**: 32-38

10. Pietrowska M, Kołodziejczyk I, Widłak P (2006): ***Mitochondrial transcription factor A is the major protein in rodent hepatocytes that recognizes DNA lesions induced by N-acetoxy-acetylaminofluorene.***
Extracts from rodent liver cells contain an abundant protein that recognizes DNA adducts induced by the chemical carcinogen N-acetoxy-acetylaminofluorene (AAAF). This protein also has a strong affinity for DNA damaged by cisplatin (DDP), but not by benzo(a)pyrene diolepoxide or UV-radiation, and has been termed AAAF/DDP-DDB. Here we purified this protein from rat tissue and analyzed it by mass spectrometry and identified it as mitochondrial transcription factor A (TFAM). Experiments with bacterially expressed recombinant TFAM confirmed its high affinity for DNA damaged by AAAF. Assuming its abundance and specificity for AAAF induced lesions, TFAM may significantly impede recognition and repair of DNA adducts induced by AAAF and other derivatives of 2-aminofluorene. *Acta Biochim Polon* **53**: 777-782

11. Sikora E, Bielak-Żmijewska A, Magalska A, Piwocka K, Mosieniak G, Kalinowska M, Widłak P, Cymerman I A, Bujnicki J M (2006): ***Curcumin induces caspase-3-dependent apoptotic pathway but inhibits DNA fragmentation factor 40/caspase-activated DNase endonuclease in human Jurkat cells.***
Curcumin is a natural pigment that has been shown to induce cell death in many cancer cells; however, the death mode depends on the cell type and curcumin concentration. Here we show that, in Jurkat cells, 50 micromol/L curcumin severely lowers cell survival and induces initial stage of chromatin condensation. It also induces caspase-3, which is sufficient to cleave DFF454/ICAD, the inhibitor of DFF40/CAD endonuclease. However, the release of DFF40/CAD from its inhibitor does not lead to oligonucleosomal DNA degradation in curcumin-treated cells. Moreover, curcumin treatment protects cells from UVC-induced oligonucleosomal DNA degradation. In biochemical experiments using recombinant DFF activated with caspase-3, we show that curcumin inhibits plasmid DNA and chromatin degradation although it does not prevent activation of DFF40/CAD endonuclease after its release from the inhibitor. Using DNA-binding assay, we show that curcumin does not disrupt the DNA-DFF40/CAD interaction. Instead, molecular modeling indicates that the inhibitory effect of curcumin on DFF40/CAD activity results from curcumin binding to the active center of DFF40/CAD endonuclease. *Mol Cancer Ther* **5**: 927-934

12. Widłak P, Garrard W T (2006): ***The apoptotic endonuclease DFF40/CAD is inhibited by RNA, heparin and other polyanions.***
DFF40/CAD, the major apoptotic nuclease, is specific for double-stranded DNA. However, RNA and single-stranded DNA, though not substrates for the enzyme, compete with double-stranded DNA and inhibit its cleavage by the nuclease. In addition, other anionic polymers, like poly-glutamic acid and heparin also inhibit DFF40/CAD, the latter one being highly effective at nanomolar concentrations. The inhibitory poly-anions bind to the nuclease and impair its ability to bind double-stranded DNA. We propose that such poly-anions bind to the positively charged surface formed by alpha4 helices of the DFF40/CAD homodimer. This surface has been proposed recently to bind to either the major groove of DNA or poly (ADP-ribose), another inhibitor of the nuclease. *Apoptosis* **11**: 1331-1337.

13. Widłak P, Pietrowska M, Łanuszewska J (2006): ***The role of chromatin proteins in DNA damage recognition and repair.***
The structure of chromatin is the major factor determining the rate and efficiency of DNA repair. Chromatin remodeling events such as rearrangement of nucleosomes and higher order chromatin structures are indispensable features of repair processes. During the last decade numerous chromatin proteins have been identified that preferentially bind to different types of DNA damage. The HMGB proteins, which preferentially interact with DNA intrastrand crosslinks induced by cisplatin, are the archetypal example of such proteins. Several hypothetical models have been proposed describing the role of such damage-binding chromatin proteins. The damage shielding model postulates that binding of chromatin proteins to damaged DNA might disturb damage recognition by repair factors and impair its removal. Alternatively, the damage-recognition/signaling model proposes that the binding of specific chromatin proteins to damaged DNA could serve as a hallmark to be recognized by repair proteins. Additionally, the binding of specific chromatin proteins to damaged DNA could induce chromatin remodeling at the damage site and indirectly affect its repair. This paper aims to critically review current experimental data in relation to such possible roles of chromatin proteins. *Histochem Cell Biol*. **125**: 119-126.

14. Widlak P, Garrard WT (2006): ***Unique features of the apoptotic endonuclease DFF40/CAD relative to micrococcal nuclease as a structural probe for chromatin.***
The gold standard for studies of nucleosomal chromatin structure for the past 30 years has been the enzyme micrococcal nuclease (MNase). During the course of our studies on the elucidation of the mechanism of action of the apoptotic nuclease DFF40/CAD on naked DNA and chromatin substrates, it became clear that this enzyme is superior in certain respects to MNase for studying several aspects of chromatin structure. Here we review our published results supporting this statement. Relative to MNase, we have found that DFF40/CAD has the following properties: (i) it does not cut within nucleosomes to generate subnucleosomal DNA fragments; (ii) it is more specific for the linker regions between nucleosomes; (iii) it lacks exonuclease activity; (iv) it is specific for double-stranded DNA and makes exclusively double-stranded breaks; and (v) it attacks histone-H1-containing chromatin more efficiently. We therefore recommend the following uses for DFF40/CAD for chromatin research: nucleosome isolation, chromatin-remodeling assays, repeat length measurements, and nucleosome-positioning assays along specific sequences. *Biochem Cell Biol*. **84**: 405-410.

Laboratory of Molecular Diagnostics and Functional Genomics

and

Laboratory of Therapy Planning and Molecular Imaging

within

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The research projects are focused on studies of genetic predisposition to neoplasms of endocrine glands and other hereditary neoplasms, functional genomics of endocrine cancer, molecular cancer markers and their clinical applications, molecular radiobiology of thyroid cancer, targeted therapy of endocrine cancer. We perform translational research, both in molecular diagnostics and molecular therapy of thyroid cancer, other endocrine-related cancers and adenomas. We analyze hereditary/somatic mutations and single nucleotide polymorphisms, changes in gene expression at single gene and genomic level, apply bioinformatic methods to study cancer transcriptome, to derive novel clinically relevant molecular markers and to analyze the biology of endocrine-related cancer. The laboratory

carries out own research and provides molecular services as core laboratory (oligonucleotide microarray analysis, real time Q-PCR , sequencing)

Specific topics include: gene expression profiling in tumors (thyroid, parathyroid, adrenals, pituitary, breast, lung, pancreas, head and neck); genetic predisposition to medullary thyroid carcinoma and pheochromocytoma, genetic presisposition to differentiated thyroid cancer; somatic mutations and chromosomal rearrangements in papillary and medullary thyroid carcinomas; the role of thyroglobulin as an early marker of differentiated thyroid carcinoma; novel targeted therapies in the treatment of differentiated and medullary thyroid cancer.

Clinical research of the Department is focused on application of molecular techniques into clinical practice of endocrine oncology and development of new diagnostic and therapeutic methods. In medullary thyroid cancer patients new targeted therapy is under evaluation. There is an ongoing project on clinical application of radiolabelled somatostatin analogues in treatment of endocrine malignancies. In differentiated thyroid cancer study on rh-TSH in treatment of disseminated disease is continued and evaluation of long-term side effects of radionuclide therapy was initiated.

Cooperation with other centers. The Laboratory acts on two levels:

1. "Core laboratory" which includes 3 units providing molecular services: microarray laboratory, QPCR laboratory and sequencing laboratory.
2. Cooperation with:
   - Professor Andrzej Świerniak and Professor Andrzej Polański, Institute of Automatic Control, Silesian Technical University
   - Professor Mike Atkinson, GSF -National Research Center for Environment and Health, Germany and Dr. Gerry Thomas, South West Wales Cancer Institute, Swansea, Walia (common project FP6/STREPS: Genetic component of the low dose risk of thyroid cancer);
   - Professor Ralf Paschke, University of Leipzig, Germany (common study on transcriptome of thyroid nodules, application of new molecular markers of follicular carcinoma);
   - Professor Martin Schlumberger, Institute Gustave-Roussy, France (targeted therapy trials)
   - Professor Massimo Santoro, Dipartimento di Biologia e Patologia Cellulare e Molecolare, Neapol (functional genomic of thyroid cancer)
   - Professor Christopher Reiners, Klinik und Poliklinik für Nuklearmedizin Universitätsklinikum Würzburg (radioiodine therapy, childhood thyroid carcinoma)
   - Professor Richard Baum, Zentralklinik Bad Berka, Germany (targeted therapy of neuroendocrine tumors)
   - Professor Andrzej Januszewicz, Institute of Cardiology, Warsaw (genetic predisposition to pheochromocytoma)
   - Professor Jacek Jassem, Medical University of Gdańsk (gene expression profile of lung cancer)
   - Professor Paweł Lange and Dr. Marek Olakowski (gene expression profile of pancreatic cancer)
   - Dr. Tomasz Bednarczuk, Department of Endocrinology, M. Mossakowski Medical Research Center, Polish Academy of Sciences, Warsaw and Dr Y. Hiromatsu, Department of Endocrinology and Metabolism, Kurume University School of Medicine, Fukuoka, Japan (multigene predisposition to Graves-Basedow disease)
   - Dr. Tatiana Gierek and Dr. Jarosław Markowski, Clinic of Laryngology, Silesian Medical University, Katowice (gene expression profile of larynx cancer)
   - Professor Tomasz Kręcicki, Clinic of Otolaryngology, Medical Academy of Wroclaw (molecular profile of head and neck carcinomas)

   The major projects performed in cooperation:
   - ❖ Sixth Research and Technological Development Framework Programme of the European Commision "Generisk-T"
   - ❖ Research project nr PBZ-MNiI-2/1/2005. The analysis of interferences in cell transduction pathways in cancer pathogenesis with the application of methods of integrating genomics.
   - ❖ Research project nr 2PO 5B 085 26. The frequency of incidence of SDHB, SDHD and other gene mutations in patients with pheochromocytoma based on national register.

Selected Papers:

1. Jarząb B, Wiench M, Fujarewicz k, Simek K, Jarząb M, Oczko-Wojciechowska M, Włoch J, Czarniecka A, Chmielik E, Lange D, Pawlaczek A, Szpak S, Gubała E, Świerniak A (2005): ***Gene expression profile of papillary thyroid cancer: sources of variability and diagnostic implications.***
   The study looked for an optimal set of genes differentiating between papillary thyroid cancer (PTC) and normal thyroid tissue and assessed the sources of variability in gene expression profiles. The analysis was done by oligonucleotide microarrays (GeneChip HG-U133A) in 50 tissue samples taken intraoperatively from 33 patients (23 PTC patients and 10 patients with other thyroid disease). In the initial group of 16 PTC and 16 normal samples, we assessed the sources of variability in the gene expression profile by singular value decomposition which specified three major patterns of variability. The first and the most distinct mode grouped transcripts differentiating between tumor and normal tissues. Two consecutive modes contained a large proportion of immunity-related genes. To generate a multigene classifier for tumor-normal difference, we used support vector machines-based technique (recursive feature replacement). It included the following 19 genes: DPP4, GJB3, ST14, SERPINA1, LRP4, MET, EVA1, SPUVE, LGALS3, HBB, MKRN2, MRC2, IGSF1, KIAA0830, RXRG, P4HA2, CDH3, IL13RA1, and MTMR4, and correctly discriminated 17 of 18 additional PTC/normal thyroid samples and all 16 samples published in a previous microarray study. Selected novel genes (LRP4, EVA1, TMPRSS4, QPCT, and SLC34A2) were confirmed by Q-PCR.Our results prove that the gene expression signal of PTC is easily detectable even when cancer cells do not prevail over tumor stroma. We indicate and separate the confounding variability related to the immune response. Finally, we propose a potent molecular classifier able to discriminate between PTC and nonmalignant thyroid in more than 90% of investigated samples. *Cancer Res* **65**: 1587-1597

2. Eszlinger M, Wiench M, Jarzab B, Krohn K, Beck M, Lauter J, Gubała E, Fujarewicz K, Świerniak A, Paschke R.(2006): ***Meta- and reanalysis of gene expression profiles of hot and cold thyroid nodules and papillary thyroid carcinoma for gene groups.***
   CONTEXT: There are an increasing number of studies analyzing gene expression profiles in various benign and malignant thyroid tumors. This creates the opportunity to validate results obtained from one microarray study with those from other data sets. This process requires rigorous methods for accurate comparison. OBJECTIVE: The ability to compare data sets derived from different Affymetrix GeneChip generations and the influence of intra- and interindividual comparisons of gene expression data were evaluated to build multigene classifiers of benign thyroid nodules to verify a previously proposed papillary thyroid carcinoma (PTC) classifier and to look for molecular pathways essential for PTC oncogenesis. METHODS: Gene expression profile data sets from autonomously functioning and cold thyroid nodules and from PTC were analyzed by support vector machines. GenMAPP analysis was used for PTC data analysis to examine the expression patterns of biologically relevant gene sets. RESULTS: Only intraindividual reference samples allowed the identification of subtle changes in the expression patterns of relevant signaling cascades, such as the MAPK pathway in PTC. Using an artificial intelligence approach, the autonomously functioning and cold thyroid nodule multigene classifiers were derived and evaluated by cross-comparisons. CONCLUSION: We recommend defining classifiers within one generation of gene chips and subsequently checking them across different array generations. Using this approach, we have demonstrated the specificity of a previously reported PTC classifier on an independent collection of benign tumors. Moreover, we propose multigene classifiers for different types of benign thyroid nodules. *J Clin Endocrinol Metab* **91**: 1934-42

3. Woźniak A, Wiench M, Olejniczak A, Włoch J, Łachiński A, Lange D, Olczyk T, Jarząb B, Limon J (2005): ***Loss of heterozygosity in 73 human thyroid tumors.***
   OBJECTIVES: The aim of the study was to establish the LOH frequency of selected polymorphic markers in different histological types of thyroid tumors: 18 colloid goiters (CG), five follicular adenomas (FA), nine follicular carcinomas (FTC), 40 papillary carcinomas (PTC), and one anaplastic carcinoma (ATC). For PTC, tumors negative for RET/PTC rearrangements were preferred. METHODS: LOH studies were performed using 14 highly polymorphic markers previously described as frequently lost in thyroid tumors. RESULTS: In 20 cases (27%) the loss of at least one marker was found. No difference between the frequency of the LOH in FTC and PTC tumors was revealed (33% v. 33%). No differences between histopathological subtypes of PTC in LOH were found. Papillary thyroid carcinomas showed a tendency to higher LOH frequency from patients older than 45 years of age compared to younger ones (9/23 v. 4/17)

although it was not statistically significant. CONCLUSIONS: We conclude that papillary thyroid cancers, particularly those diagnosed in patients older than 45 years of age, do exhibit LOH at least with the same frequency as follicular cancers. This increased number of LOH events may contribute to the clinical aggressiveness of cancer in older patients. *Neuro Endocrinol Lett* **26**: 521-5

4. Handkiewicz-Junak D, Banasik T, Kołosza Z, Roskosz J, Kukulska A, Puch Z, Jarząb B (2006): ***Risk of malignant tumors in first-degree relatives of patients with differentiated thyroid cancer – a hospital based study.***
In presented study the risk of incidence of familial differentiated thyroid cancer as well as the risk of other malignant tumors in families of DTC patients was evaluated. 999 patients with differentiated thyroid cancer and 825 persons without any history of malignant disease were evaluated on the occurrence of malignant neoplasm within their families. Information about 6614 first degree relatives of DTC index patients and 4939 first degree relatives of control persons were recorded. The incidence of cancers at various sites was compared between first-degree relatives of index patients and control persons and odds ratio with 95% confidence intervals (CI) were calculated for thyroid cancer and other cancer sites. Within 999 families of thyroid cancer index patients 23 families with more than one case of DTC were found. The risk of the development of thyroid cancer in the first degree was 6 (95% CI 1.8-19) times greater in the index group than in the control group. No increased risk for development of other malignancies was observed. Results of our study confirm previous reports of increased risk of thyroid cancer in first-degree relatives of differentiated thyroid cancer patients. However, the relatively small number of first-degree relatives affected with thyroid cancer (24/6614) does not justify at present any screening in the first-degree relatives of patients affected with differentiated thyroid cancer. Simultaneously, no increased risk of other malignant neoplasm was observed in the differentiated cancer families. *Neoplasma* **53**: 67-72

5. Kuryłowicz A, Kula D, Płoski R, Skórka A, Jurecka-Lubieniecka B, Żebracka J, Steinhof-Radwańska K, Hasse-Lazar K, Hiromatsu Y, Jarząb B, Bednarczuk T (2005): ***Association of CD40 gene polymorphism (C-1T) with susceptibility to and phenotype of Graves' disease.***
OBJECTIVE: Recently, a functional polymorphism in the CD40 gene at position -1, C to T change (C-1T) has been identified and the C/C genotype has been reported to be associated with Graves' disease (GD). DESIGN: We performed a case-control, replication study on 556 patients with GD and 611 healthy subjects in a Polish population. Furthermore, we analyzed the distribution of CD40 genotypes in subgroups of patients with GD divided according to age of onset, gender, family history, tobacco smoking, ophthalmopathy, and genetic parameters (CTLA4 49G, PTPN22/LYP 1858T or HLA-DRB1*03 alleles). RESULTS: Although the frequency of C/C genotype was increased in GD compared to controls, the difference was not significant (60.5% versus 55.8%, p = 0.062, odds ratio [OR] = 1.21, 95% confidence interval [CI]: 0.96-1.53). Because our study was underpowered to detect such a modest association, we performed a meta-analysis with the data from previous studies. The combined OR for the C/C genotype as a risk factor for GD was 1.22 (95% CI: 1.08-1.38, p = 0.001). We failed to find an interaction between CD40 genotypes and other GD susceptibility alleles. No significant genotype-phenotype associations were found. CONCLUSIONS: Our results support the notion that CD40 C-1T polymorphism has a modest effect on genetic susceptibility to sporadic GD. *Thyroid* **15**: 1119-1124

6. Kula D, Bednarczuk T, Jurecka-Lubieniecka B, Polańska J, Hasse-Lazar K, Jarząb M, Steinhof-Radwańska K, Hejduk B, Żebracka J, Kuryłowicz A, Bar-Andziak E, Stęchły T, Pawlaczek A, Gubała E, Krawczyk A, Szpak-Ulczok S, Nauman J, Jarząb B. (2006): ***Interaction of HLA-DRB1 alleles with CTLA-4 in the predisposition to Grave's disease: the impact of DRB1*07.***
OBJECTIVE: To study interactions between the two most widely confirmed Graves' disease (GD) loci: HLA-DRB1 and CTLA-4. HLA-DRB1*03 (risk allele) and DRB1*07 (protective allele) were analyzed in this aspect, the linked TNF G(-308)A polymorphism was also considered. DESIGN: A case-control study of 429 patients with GD compared to 308 healthy subjects. The impact of genes and their interactions were analyzed by stepwise logistic regression. RESULTS: The independent effects of DRB1*03 and DRB1*07 were confirmed in our study both by stratification studies and logistic regression. CTLA-4 did not appear to be associated with GD when the interactions with other genes were considered. By logistic regression we observed a significant interaction between DRB1*07 and CTLA-4 and revealed that CTLA-4 49G attenuated the DRB1*07-related protection, the effect noticed also in three-way stratification studies. We confirmed that the TNF G(-308)A polymorphism is only a marker of the DRB1 status. CONCLUSION:

Our results stress the importance of complex gene interactions in the multigene predisposition to GD. The interactions between two predisposing loci, DRB1 and CTLA-4, are exerted rather by DRB1*07 than DRB1*03 allele: CTLA-4 acts via switching off the protective DRB1*07 influence, whereas the effect of DRB1*03 is independent. *Thyroid* **16**: 447-53

7. Jarząb B, Handkiewicz-Junak D, Włoch J. (2005): ***Juvenile differentiated thyroid carcinoma and the role of radioiodine in its treatment: a qualitative review***.
Well under 15% of differentiated thyroid carcinoma (DTC) is diagnosed at < or =18 years of age. The population is heterogenous and the differences between prepubertal children and pubertals and adolescents are to be considered. Although very little has been reported on children with sporadic DTC under the age of 10 years, juvenile DTC has at least some undeniable differences with adult DTC: (1) larger primary tumor at diagnosis; (2) metastatic pattern and features, namely: (a) greater prevalence of neck lymph node and distant metastases at diagnosis, (b) lungs almost the sole distant metastatic site, (c) pulmonary metastases nearly always functional; (3) closer-to-normal and more frequent sodium-iodide symporter (NIS) expression; and (4) higher recurrence rate but longer overall survival. These differences are especially distinct in prepubertal children. The goals of primary treatment of juvenile DTC are to eradicate disease and extend not only overall, but recurrence-free survival (RFS). Extending RFS is itself a desirable goal in children because it improves quality-of-life, alleviates anxiety during psychologically formative years, reduces medical resource consumption, and may increase overall survival. Primary treatment of DTC generally comprises a combination of surgery, radioiodine ((131)I) ablation, and thyroid hormone therapy applied at varying levels of intensity. Therapeutic decision-making must rely on retrospective adult and/or pediatric outcome studies and on treatment guidelines formulated mostly for adults. Differences between juvenile and adult DTC and physiology dictate distinct treatment strategies for children. We, and many others, advocate a routine intensive approach because of the more advanced disease at diagnosis, propensity for recurrence, and greater radioiodine responsiveness in children, as well as published evidence of significant survival benefits, especially regarding RFS. This intensive approach consists of total thyroidectomy and central lymphadenectomy in all cases, completed by modified lateral lymphadenectomy when necessary and followed by radioiodine administration. However, absence of prospective studies and of universal proof of overall cause-specific survival benefits of this approach have led some to propose more conservative strategies. Most European centers give radioiodine ablation to the vast majority of juvenile DTC patients. Ablation seeks to destroy any residual cancer, including microfoci, as well as healthy thyroid remnant. Large studies have documented the procedure to decrease cause-specific death rates and, in children, to significantly lessen locoregional recurrence rates (by factors of 2-11) independent of the extent of surgery. There is universal agreement on treating inoperable functional metastases with large radioiodine activities. Treatment is especially effective in small tumor foci up to 1 cm in diameter, and should be administered every 6-12 months until complete response, loss of functionality, or attainment of cumulative activities between 18.5-37 GBq (500-1000 mCi). Radioiodine therapy is generally safe. Short-term side effects include nausea and vomiting (more frequent in children than in adults), transient neck pain and edema, sialadenitis (<5% incidence), mild myelosuppression (approximately 25%), transient impairment of gonadal function both in females and males (sperm quality in boys), or nasolacrimal obstruction (approximately 3%), with most cases generally being asymptomatic-moderate, self-limiting, or easily prevented or treated. If pregnancy is ruled out before each (131)I administration, and conception avoided in the year afterward, radioiodine therapy appears not to impair fertility. However, therapeutic (131)I carries a small but definite increase in cancer risk, particularly in the salivary glands, colon, rectum, soft tissue and bone. To better guide primary treatment, different therapeutic combinations should be prospectively compared using RFS as the primary endpoint. Efforts also should be made to identify molecular signatures predicting recurrence, metastasis and mortality. *Endocr Relat Cancer* **12**: 773-803

8. Luster M, Lippi F, Jarzab B, Perros P, Lassman M, Reiners C, Pacini F (2005): ***rhTSH-aided radioiodine ablation and treatment of differentiated thyroid carcinoma: a comprehensive review.***
Traditionally, withdrawal of thyroid hormone has been used to attain the increase in serum TSH concentrations that are believed to optimize the trapping and retention of radioiodine for diagnostic procedures, thyroid remnant ablation and treatment of patients with differentiated thyroid cancer (DTC). However, withdrawal frequently causes clinical hypothyroidism, with resultant cognitive impairment, emotional dysfunction, physical discomfort, health risks in patients who are elderly, frail or have concomitant illness, and impaired quality of life and ability to work. Recombinant human TSH (rhTSH) was developed to provide TSH stimulation without withdrawal of thyroid hormone and the associated morbidity. rhTSH has been approved as an adjunct for diagnostic procedures in patients with DTC, but is

currently an experimental aid in thyroid remnant ablation and the treatment of thyroid tumours. In the period 1997-2004, nearly 30 medical centres worldwide have reported on almost 400 patients with DTC who were given rhTSH in preparation for radioiodine ablation of thyroid remnants or treatment of local tumours of metastatic disease. We have analysed and summarized the findings reported in this literature. *Endocrine-Related Cancer* **12**: 49-64

9. Brabant G, Beck-Peccoz P, Jarząb B, Laurberg P, Orgiazzi J, Szabolcs I, Weetman AP, Wiersinga WM (2006): ***Is there a need to redefine the upper normal limit of TSH?***
Mild forms of hypothyroidism--subclinical hypothyroidism--have recently been discussed as being a risk factor for the development of overt thyroid dysfunction and for a number of clinical disorders. The diagnosis critically depends on the definition of the upper normal limit of serum TSH as, by definition, free thyroxine serum concentrations are normal. Cut-off levels of 4-5 mU TSH/l have been conventionally used to diagnose an elevated TSH serum concentration. Recent data from large population studies have suggested a much lower TSH cut-off with an upper limit of 2-2.5 mU/l but application of strict criteria for inclusion of subjects from the general population studies aiming at assessing TSH reference intervals (no personal or family history of thyroid disease, no thyroid antibodies and a normal thyroid on ultrasonography) did not result in an unequivocal upper limit of normal TSH at 2.0-2.5 mU/l. When summarizing the available evidence for lowered upper TSH cut-off values and their potential therapeutic implications there is presently insufficient justification to lower the upper normal limit of TSH and, for practical purposes, it is still recommended to maintain the TSH reference interval of 0.4-4.0 mU/l. Classifying subjects with a TSH value between 2 and 4 mU/l as abnormal, as well as intervening with thyroxine treatment in such subjects, is probably doing more harm than good. *Eur J Endocrinol* **154**: 633-7

10. Wygoda Z, Kula D, Bierzyńska-Macyszyn G, Larysz D, Jarząb M, Właszczuk P, Bażowski P, Wojtacha M, Rudnik A, Stępień T, W, Etmańska A, Składowski K, Tarnawski R, Kokocińska D, Jarząb B (2006): ***Use of monoclonal anti-EGFR antibody in the radioimmunotheraphy of malignant gliomas in the context of EGFR expression in grade III and IV tumors.***
We investigated the putative benefits of simultaneous teleradiotherapy and anti-epidermal growth factor receptor (EGFR) 125I monoclonal antibody (MAb) 425 radioimmunotherapy, when applied after neurosurgery in high-grade gliomas, over teleradiotherapy alone. In comparison to previous studies which have reported good results with this type of radioimmunotherapy, we advanced the adjuvant radioimmunotherapy step, that is, gave it during, not after, teleradiotherapy. The randomized prospective study examined two groups: simultaneous postoperative teleradiotherapy and radioimmunotherapy (TRT + RIT; eight patients) versus teleradiotherapy alone (TRT; 10 patients). Patients who after primary operation of grade III (6 cases) or IV glioma (12 cases), showed no or less than 2 mL of remnant tumor on post-operative magnetic resonance (MR) study and were not treated postoperatively by chemotherapy were enrolled and randomized. Anti-EGFR 125IMAb 425 RIT was started during week 4 of radiotherapy, not later than 8 weeks after neurosurgery, and was repeated three times at 1-week intervals. Total activity given was 5026 + 739 MBq/patient. The tolerance of TRT was good. No immediate side effects of concomitant anti-EGRF 125I RIT were observed. Observation showed a median total survival (as evaluated from the primary neurosurgical treatment) of 14 months (range 3.5-28 months). There was no improvement in disease-free or total survival in the group of patients treated by TRT + RIT after neurosurgery. In addition, an immunohistochemical analysis of EGFR expression in gliomas was performed in a group of 100 cases and was distinctly positive in 50% grade IV gliomas and 68% grade III gliomas. We conclude that simultaneous radiotherapy and radioimmunotherapy with anti-EGFR 125I-MAb 425 is not beneficial over radiotherapy alone in adjuvant treatment of high-grade gliomas after neurosurgery. We also recommend individual confirmation of EGFR expression in further anti-EGFR radioimmunotherapy trials. *Hybridoma* **25**: 125-32

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Laboratory of Biophysics

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The laboratory brings together physicists, biologists and chemists engaged in the studies of brain biochemistry using NMR spectroscopy. Research is focused on the studies of intact biological systems by MRS in vivo and the high resolution in vitro NMR model studies of animal brain extracts and cell lines.
The main scientific interests involve:

- Application of ^{1}H MR in vivo spectroscopy and multivariate statistical methods (PCA, PLS-DA, OSC) in the studies of brain glial tumor metabolic profiles and uncertain zone analyses.
- Detection of early and late metabolic effects of brain irradiation using ^{1}H MRS *in vivo*, ^{1}H NMR *in vitro* of C6 cell line and multivariate statistical methods (PCA, PLS-DA, OSC).
- Investigation of brain metabolism disturbances in smokers and in patients with lung cancers – multivariate statistical analyses (PCA, PLS-DA, OSC) of NMR data and comparative animal model studies.
- Evaluation of usefulness of ^{1}H MRS *in vivo* in diagnostics of progressive encephalopathies in children.

Laboratory of Dosimetry and Quality Control in Radiotherapy and Rentgenodiagnostics

Head: Andrzej Orlef, Ph.D. (aorlef@io.gliwice.pl)

The focus of the research in this laboratory is the clinical implementation of improved treatment and verification methods, like portal imaging and in vivo dosimetry.
The new area of studies is 3D polymer gel dosimetry using MRI readout methods.

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Selected Papers:

1. Sokół M (2005): ***High resolution NMR Studies of Brain Tumors.*** in: *Nuclear Magnetic Resonance Spectroscopy in the study of neoplastic tissue*, red. M.R. Tosi and V. Tugnoli , *Nova Science Publishers*, Inc., New York, USA, 2005
2. Tarnawski R, Sokół M, Blamek S (2005): ***In vivo MRS of human brain tumors.*** in: *Nuclear Magnetic Resonance Spectroscopy in the study of neoplastic tissue*, red. M.R. Tosi and V. Tugnoli , *Nova Science Publishers*, Inc., New York, USA, 2005
3. Matulewicz Ł, Pohl A, Sokół M, Białecki R (2005): ***Principal component analysis of in vivo 1H NMR spectra from patients postoperatively irradiated for malignant gliomas.*** *Magnetic Resonance Materials in Physics, Biology and Medicine* **18**:176-307
4. Cichoń A, Sokół M, Cichoń T, Gibas M (2006): ***Brain metabolism disturbances in case of lung diseases – in vitro NMR studies of mice brain extracts.*** *Pol J Environ Stud* **15**: 44-46
5. Matulewicz L, Cichon A, Jurkowski M, Przybyszewski WM, Gibas M, Sokół M (2006): ***Response to Do dose of ionizing radiation of C6 glioma cell line measured by high resolution 1H NMR spectroscopy.*** *Polish J Environ Stud* **15:**187-190.
6. Matulewicz Ł, Cichoń A, Michnik A, Sokół M (2006): ***Statistical analysis of spectroscopic pre-and postirradiation normal-appearing brain tissue.*** *Magnetic Resonance Materials in Physics, Biology and Medicine* **19**: 258-259
7. Matulewicz Ł, Sokół M, Michnik A, Wydmański J (2006): ***Long – term normal – appearing brain tissue monitoring after irradiation using proton mr spectroscopy in vivo. Statistical analysis of large group of patients.***
   PURPOSE: The aim of this study was to detect the non-neoplastic white-matter changes vs. time after irradiation using 1H nuclear magnetic resonance (NMR) spectroscopy in vivo. METHODS AND MATERIALS: A total of 394 1H MR spectra were acquired from 100 patients (age 19-74 years; mean and median age, 43 years) before and during 2 years after radiation therapy (the mean absorbed doses calculated for the averaged spectroscopy voxels are similar and close to 20 Gy). RESULTS: Oscillations were observed in choline-containing compounds (Cho)/creatine and phosphocreatine (Cr), Cho/N-acetylaspartate (NAA), and center of gravity (CG) of the lipid band in the range of 0.7-1.5 ppm changes over time reveal oscillations. The parameters have the same 8-month cycle period; however the CG changes precede the other by 2 months. CONCLUSIONS: The results indicate the oscillative nature of the brain response to irradiation, which may be caused by the blood-brain barrier disruption and repair processes. These oscillations may influence the NMR results, depending on the cycle phase in which the NMR measurements are performed in. The earliest manifestation of radiation injury detected by magnetic resonance spectroscopy is the CG shift. *Int J Radiat Oncol Biol Phys* **66**: 825-832
8. Matulewicz L, Sokol M, Wydmanski J, Hawrylewicz L (2006): ***Could lipid CH2/CH3 analysis by in vivo 1H MRS help in differentiation of tumorrecurrence and post-radiation effects?*** *Folia Neuropathol* **44**:116-24.
9. Skrzypek D, Szymańska B, Kovala-Demertzi D (2006): ***Synthesis and spectroscopic studies of Co(II) complexes with pipemidic acid.*** *Pol J Environ Stud* **15**: 97-99
10. Skrzypek D, Szymańska B, Kovala-Demertzi D, Wiecek J, Talik E, Demertzis MA (2006): ***Synthesis and spectroscopic studies of Iron(III) complex with quinolone family member (pipemidic acid).*** *J Phys Chem Solids* **67**: 2550-2558
11. Szymańska B, Skrzypek D, Kovala-Demertzi D, Staninska M, Demertzis (2006): **Synthesis and spectroscopic studies of Copper(II) and Manganese(II) and complexes with pipemidic acid.** Spectrochimica Acta Part A **63**: 518-523.

Department of Cancer Epidemiology

The Department covers the following activities:

Cancer epidemiology studies

The analytical and descriptive studies are focused on:

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

The Regional Silesia Cancer Registry (RSCR)

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

The Bio-bank (in organization since September 2004)

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

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

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The Bio-bank (in organization):
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Selected Papers:

1. Banasik T R, Kołosza Z, Zemła B F P (2005): ***Trendy czasowe umieralności na nowotwory złośliwe gruczołu krokowego w populacji Górnego Śląska***. *Medycyna Środowiskowa* **8**: 109-116

2. Kennedy D O, Agrawal M, Shen J, Terry M B, Zhang E F, Senie R T, Motykiewicz G, Santella R M (2005): ***DNA repair capacity of lymphoblastoid cell lines from sisters discordant for breast cancer.***
   ACKGROUND: Interindividual differences in DNA repair capacity may influence cancer risk. We tested whether the nucleotide excision repair pathway was deficient in breast cancer case patients by analyzing sister pairs. METHODS: Cell lines derived from sisters discordant for breast cancer (137 families containing 158 case patients and 154 control sisters) were obtained from the Metropolitan New York Registry of Breast Cancer Families. Lymphoblastoid cells were treated with benzo[a]pyrene diolepoxide (BPDE) for 30 minutes and were either harvested immediately or were washed and cultured in complete medium for 4 hours to allow DNA repair. Immunofluorescence using a polyclonal anti-BPDE-DNA primary antibody was used to quantify BPDE-DNA adducts. Percent DNA repair capacity was calculated from the difference between staining immediately after treatment minus that after 4 hours of repair, divided by the initial damage and was categorized into quartiles based on control values. Odds ratios and 95% confidence intervals (CIs) were calculated using conditional logistic regression models adjusted for age at blood donation, body mass index, and smoking. Statistical tests were two-sided. RESULTS: Mean percent DNA repair capacity was lower in breast cancer case patients than in control subjects (difference = 8.6, 95% CI = 4.3 to 13.8, P = .001). Using the quartile with the highest percent DNA repair capacity as the referent group, adjusted odds ratios of breast cancer increased from 1.23 (95% CI = 0.57 to 2.65) to 2.38 (95% CI = 1.17 to 4.86) to 2.99 (95% CI = 1.45 to 6.17) (P(trend) = .002) as DNA repair capacity decreased. CONCLUSIONS: Deficient DNA repair capacity is associated with increased breast cancer risk. *J Natl Cancer Inst* **97**: 127-132

3. Kołosza Z, Banasik T R, Zemła B F P (2005): ***Nowotwory złośliwe w województwie śląskim w 2002 roku.*** Wyd. Zakład Epidemiologii Nowotworów Centrum Onkologii - Instytut im. Marii Skłodowskiej-Curie Oddział w Gliwicach. Gliwice 2005.

4. Kołosza Z, Banasik T R, Zemła B FP (2006): ***Nowotwory złośliwe w województwie śląskim w 2003 roku.*** Wyd. Zakład Epidemiologii Nowotworów Centrum Onkologii - Instytut im. M. Skłodowskiej-Curie Oddział w Gliwicach. Gliwice 2006.

5. Kołosza Z, Banasik T R, Zemła B P F (2006): ***Nowotwory złośliwe w województwie śląskim w 2004 roku.*** Wyd. Zakład Epidemiologii Nowotworów Centrum Onkologii - Instytut im. M. Skłodowskiej-Curie Oddział w Gliwicach. Gliwice 2006.

6. Kołosza Z, Banasik T, Juszko-Piekut M, Zych-Sowa J, Zemła B P F (2006***): Trendy umieralności na nowotwory złośliwe piersi w populacji kobiet śląskich, 1975-2002.*** *Medycyna Środowiskowa* **9**: 9-17

7. Włodarczyk-Marciniec B, Kołosza Z (2006): ***Analiza zachorowalności na raki tarczycy wśród śląskich kobiet w latach 1990-2001.*** *Medycyna Środowiskowa* **9**: 19-25

8. Zieliński G, Zemła B, Harazim B, Malinowska J (2006): ***Incidence of leukemia and exposure of population to 50 Hz magnetic fields In the housing environment of cities in Silesian Voivodeship.*** *Polish J Environ Stud* **15**: 1723-1725