

TUMOR HYPOXIA AND MALIGNANT PROGRESSION

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The current problems of malignant growth biology, in particular the molecular background of the specific microenvironment of tumor cells and their interaction with stromal cells, which mediates the behavior of tumors and the tumor–host interrelationship were the subject of the International Conference entitled “Tumor Hypoxia and Malignant Progression”, a meeting held at the House of Scientists of the NAS of Ukraine in Kyiv, Ukraine, October 1st to 4th, 2008. The meeting was hosted by the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine (IEPOR), and was dedicated to the 90th Anniversary of the National Academy of Sciences of Ukraine. Over the last years, scientists have focused extensively on the problem of tumor hypoxia as a factor promoting tumor progression. It is known that hypoxia, as a constituent of the tumor cell microenvironment as well as aerobic glycolysis, are important features of malignant tumors. The direct correlation between high levels of hypoxia and tumor aggressiveness has been shown in numerous studies. Therefore, hypoxia is regarded as a factor of unfavorable prognosis. There is a number of different methods available for the evaluation of the level of hypoxia, some of which are being applied in the clinical setting. The stimulating impact of hypoxia and hypoxia-associated proteins on neoangiogenesis and vasculogenesis in tumor tissue has been demonstrated. Several studies have focused on the development of agents capable of blocking hypoxia-associated signaling pathways and vasculogenesis in tumor. Recently, the direct association between hypoxia-dependent signaling pathways and expression of factors that mediate inflammation in tumor tissue, in particular tumor-associated macrophages has been shown. To summarize, a better understanding of the relationships between hypoxia-associated signaling pathways, metabolic peculiarities and inflammatory factors that positively influence tumor progression may elucidate not only how the aggressive tumor phenotype is formed but also may assist in the development of new approaches for the treatment of cancer patients.

Over the last years, researchers have been seen to be taking an increasing interest in the problems of the tumor cell microenvironment, especially tumor hypoxia, which is one of the most prominent features of malignant neoplasias, distinguishing them from normal tissues. According to experimental and clinical studies, tumor hypoxia positively affects tumor progression, stimulating its aggressiveness, metastasis and resistance to therapy.

The molecular background of the specific microenvironment of tumor cells and its interaction with stromal cells in mediating the behavior of tumors and the tumor–host interrelationship were the subject of the International Conference entitled “Tumor Hypoxia and Malignant Progression”, a meeting held at the House of Scientists of the NAS of Ukraine in Kyiv, Ukraine, October 1st to 4th, 2008. The meeting was hosted by the R.E. Kavetsky Institute of Experimental Pathology, Oncology and Radiobiology of the NAS of Ukraine (IEPOR) and was dedicated to the 90th Anniversary of the National Academy of Sciences of Ukraine.

The purpose of the meeting was to discuss new results arising from research in different areas of tumor hypoxia and the microenvironment of tumor cells, in particular the molecular mechanisms of hypoxia-associated signaling pathways in primary tumors as key factors of tumor progression and strategies for the clinical application of hypoxia markers and the development of hypoxia-targeted anticancer agents.

The conference was opened by an official invitation from the Academy Secretary of the Branch of Biochemistry, Physiology and Molecular Biology of the

NAS of Ukraine, academy member Sergej V. Komissarenko, who has characterized the problem of tumor hypoxia, focusing attention on its biochemical aspects which were first studied in the fundamental investigations of O. Warburg. The importance of the meeting which allowed scientists from different countries to discuss new results and plan further strategies for their research was emphasized. Co-chairman of the International Scientific Committee Prof. P. Vaupel (Germany) and member of ESHO Board Prof. M. Horsman (Denmark), welcomed participants to the conference. On behalf of all invited lecturers, Prof. J. Pouyssegur (France) welcomed participants and guests to the meeting. All speakers emphasized the importance of the problem of the tumor cell microenvironment — in particular hypoxia — in the elucidation of the mechanisms of cancer aggressiveness and the elaboration of new approaches in cancer treatment. It was pointed out that such meetings help to stimulate cooperation between scientists who are engaged in different areas of hypoxia research as well as promoting discussions on further strategies worthy of investigation.

Tumor microenvironment and tumor microphysiology. In a foreword, the co-chairman of the conference *Sergej Osinsky (IEPOR, Ukraine)* welcomed participants of the meeting and concentrated their attention on the relevance of understanding the role of the tumor microenvironment in cancer progression for the development of novel therapeutic strategies. He also proposed the consideration of two types of microenvironment for tumor cells: the metabolic microenvironment mediated by factors of tumor microphysiology (blood flow, vascular permeability, oxygena-

tion, extracellular pH, interstitial fluid pressure, etc.), and the cellular-molecular microenvironment comprising interactions between tumor cells and non-tumor cells and the factors of the stromal compartment.

Peter Vaupel (Mainz University Medical Center, Germany) concentrated on tumor pathophysiology and in particular on the role of hypoxia as an important driving force in malignant progression. Hypoxia, as an inherent consequence of unregulated tissue growth promotes local invasion, intravasation and metastatic spread at three levels whereby this occurs in a cooperative manner: (i) on the transcriptome/proteome/metabolome level, (ii) on the genome/epigenome level and (iii) on the level of clonal selection of malignant cell populations. On the proteome/metabolome level, the changes in gene expression are coordinated mainly by HIF-1 and HIF-2: the downstream effects of HIF-activation include *cellular adaptation* (e. g., modulation of glucose metabolism through GLUT-1 and by up-regulation of key glycolytic enzymes, and overexpression of carbonic anhydrase IX (CA-IX), activation of the c-MET/HGF system, cell-cell dissociation, migration, and apoptosis protection), *local adaptation* (e. g., increased angiogenesis by up-regulation of VEGF, vasodilation), and *systemic adaptation* (e. g., increased O₂ transport capacity, EPO). New facets of this concept were presented, based on recent findings in leiomyomas (benign tumors of the uterus) that are severely and uniformly hypoxic (needle electrode measurements) with a persistent lack of expression of HIF-1 α /2 α , GLUT-1 and CA-IX, whereas leiomyosarcomas — highly malignant tumors originating from the same tissue — show a remarkably active HIF-mediated proteomic response. Although the molecular mechanisms behind these fundamental differences still remain unknown, these findings challenge the current view that HIF overexpression in malignant tumors essentially represents a physiological mechanism activated in a specific pathological microenvironment.

Michael Horsman (Aarhus University, Denmark) focused on the role of hypoxia in the interaction between radiation and vascular targeting agents, described the importance of the tumor vasculature as an attractive target for therapy and discussed two major groups of vascular targeting agents (VTAs): those preventing the development of the tumor vasculature by inhibiting various steps in the angiogenic process (angiogenesis inhibiting agents, AIAs) and those damaging the already established tumor vessels (vascular disrupting agents, VDAs). It was suggested that potential clinical applications of both VTA types would be helpful in combination with conventional treatment, especially radiation.

In fact, numerous pre-clinical studies have shown that the response of tumors to radiation can be significantly improved when animals are treated with either AIAs or VDAs. AIAs can improve the oxygenation status of tumors; and for both types of VDA, hypoxia is implicated in the mechanisms of the enhancement

of radiation response. Even so, the possible application of AIAs and VDAs is still somewhat controversial.

The improved anti-tumor responses observed when such agents are combined with radiotherapy likely reflect an additive tumor response resulting from the VDA eliminating treatment-resistant hypoxic tumor cells while radiotherapy acts against the normoxic tumor cell population. The timing of VDA therapy relative to radiation treatment is nevertheless clearly critical since VDAs can induce hypoxia resulting in a possible “double-edged sword” phenomenon when VDAs and radiation are combined.

Hypoxia and adaptation. *Jacques Pouyssegur (Nice University, France)* expanded on the mechanisms of tumor adaptation to a hypoxic and acidic microenvironment. In spite of the important role of nutrients for growing cells, oxygen sensing appears to be a central control mechanism for angiogenesis and energy metabolism. The hypoxia-signaling pathway has been examined at the forefront of nutritional control due to the peculiar dual role of hypoxia-inducible factor in this regulatory system, which can induce a vast array of gene products controlling glucose metabolism, intracellular pH, angiogenesis, cell migration and invasion. HIF is considered not only as a strong promoter of tumor growth but also as a factor capable of inducing pro-apoptotic genes leading to autophagy and cell death. It is of great importance to highlight some of the HIF-induced markers that participate in tumor resistance in a nutrient-depleted and acidic microenvironment: firstly, it was shown that two HIF-induced “BH-only”-proteins (BNIP3, BNIP3L) — in contrast to current theories — do not trigger cell death but rather, tumor cell survival by inducing autophagy; and secondly, it was shown that tumor cells acidify the extracellular milieu by expressing HIF-dependent anhydrases, CA-IX and CA-XII, resulting in a more alkaline intracellular pH that is more favorable for migration, survival and growth of cancer cells. It was proposed that special attention should be paid to some of these HIF-regulated targets in anticancer therapy.

Data concerning the inhibiting effects of ferromagnetic nanoparticles on oxygen consumption in the mitochondria of cancer cells were presented by *Nathalia Lukianova (IEPOR, Kyiv, Ukraine)*. In earlier studies, a cytotoxic effect of ferromagnetic nanoparticles on Ehrlich ascites carcinoma cells was found. Since active centers of many complexes within the mitochondrial respiratory chain contain iron, the author and colleagues investigated the influence of stabilized ferromagnetic nanoparticles (Fe₃O₄; size 40–60 nm) on tumor cell oxygen consumption and mitochondrial oxidative phosphorylation. Ferromagnetic nanoparticles at a concentration of 1.45 μ g Fe/ml and, in particular, 7.25 μ g Fe/ml, decrease mitochondrial oxygen consumption in the phosphorylation state and thus may negatively affect survival capability.

Hypoxia and biomarkers. *Catherine West (Manchester University, UK)* discussed the problem of hypoxia and gastric cancer development and prognosis

since the role of hypoxia in the etiology and prognosis of esophago-gastric cancer has not yet been studied. The data obtained to date with hypoxia-associated markers do not suggest that hypoxia plays a key role in the development and prognosis of gastro-esophageal cancer. However, conflicting data have been reported in the literature regarding the prognostic significance of HIF proteins — tumor expression has been linked — predominately with poor prognosis, but occasionally with a good prognosis.

To increase the understanding of esophago-gastric cancer biology and develop methods for determining prognosis, hypoxia-associated markers (HIF-1 α , HIF-2 α , VEGF, Epo, Epo-R, Glut-1) were studied in esophageal and gastric carcinogenesis sequences. The expression of cleaved PARP as a marker of apoptosis was also examined. The expression of all the hypoxia-associated markers studied was seen to be increased significantly from normal tissue to invasive malignancies in both carcinogenesis models. HIF-2 α was expressed late in Barrett's sequence, and was only seen in dysplasia and cancer samples. The data obtained provide some evidence to suggest that when tumor HIF expression is associated with a good prognosis it might be functioning in a pro-apoptotic tumor suppressing mode rather than being hypoxia-induced. The high expression of HIF-2 α and its late expression in the carcinogenesis models suggest that it might be worth further investigation as a therapeutic target and as a marker of disease progression in patients with Barrett's dysplasia.

Antonina Kovelskaya (IEPOR, Kyiv, Ukraine) presented results concerning the evaluation of hypoxia level by ^{31}P -NMR spectroscopy in perchloric extracts of human tumors. Since the assessment of tumor hypoxia could prove to be useful for the recruitment of patients for individualized therapy, the search for the relevant hypoxia markers is of utmost importance. Previously, the authors showed that the PME/Pi ratio is associated with the level of hypoxia in human gastric cancer as well as in cervical carcinoma. The current study was aimed at verifying whether the PME/Pi ratio in tumor tissue reflects the real state of hypoxia in neoplasia. The hypoxia level found in moderately hypoxic gastric cancer was compared with that of highly hypoxic cervical carcinomas based on ^{31}P -NMR spectroscopy data and immunohistochemical analysis of the hypoxia endogenous marker CA-IX. It was clearly shown that the PME/Pi metabolic ratio may reflect the real oxygenation state in tumor tissue. Therefore, ^{31}P -NMR spectroscopy of perchloric extracts from tumors may be used as a reliable tool for the assessment of the level of hypoxia in individual tumor surgical specimens and the PME/Pi ratio may be suitable as a hypoxia marker for ^{31}P -NMR spectroscopy *in vivo*.

Valentina Grinkevich (IEPOR, Kyiv, Ukraine) presented data on the peculiarities of angiogenesis and drug resistance in human ovarian cancer. It is known that hypoxia can stimulate microvessel formation in tumors and the expression of genes associated with drug

resistance. *GST* is one of the genes involved in tumor resistance to platinum-containing drugs. Microvessel density (MVD), VEGF and *GST* expression were immunohistochemically evaluated in ovarian serous carcinoma samples from 71 patients and compared with disease outcome. The maximum level of VEGF expression and the highest MVD were detected in G1 tumors. *GST* expression was significantly increased in G3 carcinomas. VEGF expression and MVD were substantially elevated in tumors of patients with III–IV stages as compared to those in patients with I–II stages (FIGO). The significant increase in the MVD value, VEGF and *GST* expression was also seen in tumors of patients with metastases. The increased MVD and VEGF expression have been associated with poor 5-year survival. It was concluded that *GST* expression may indicate the natural resistance of tumors and has to be taken into account before choosing chemotherapeutic regimens, and that the MVD data, VEGF and *GST* expression may be used as prognostic markers.

Maryana Shabelnik (IEPOR, Kyiv, Ukraine) presented results on the expression and autophosphorylation of protein kinases D in human gastric adenocarcinoma. It is known that serine/threonine protein kinases of the PKD family play a key role in the integration of different signaling pathways. PKD2 is the major protein kinase of the PKD family in the AGS human gastric cancer cell line. In these cells, PKD2 is activated by gastrin and is a downstream target of specific PKCs. Hypoxia-inducible factor (HIF) is a master transcriptional factor in nutrient stress signaling. HIF-1 α has been implicated in gastric tumor promotion and angiogenesis. This study was aimed at elucidating the level of expression and autophosphorylation of PKD1 and PKD2 kinases in primary samples of gastric adenocarcinoma and to clarify whether they correlate with HIF-1 α expression. It was found that not only PKD2, but also PKD1 is expressed in primary gastric cells. Adenocarcinomas were found to be heterogenous as far as the level of PKD1/2 expression and activity, and also their intracellular localization was concerned. The level of PKD1/2 expression, autophosphorylation and intracellular localization correlated directly with tumor differentiation grade: moderately differentiated tumors showed high levels of PKD1/2 expression and activation in both the cytoplasm and nucleus. Less well differentiated tumors were characterized by weak PKD1/2 immunohistochemical staining in the cytoplasm. The level of PKD1/2 expression and its activity did not correlate with HIF1 α .

Maria Soldatkina (IEPOR, Kyiv, Ukraine) presented results on human defensins that are endogenous multifunctional peptide antibiotics produced by cells of various histologies. There is growing evidence that apart from antibacterial, fungicidal and antiviral activities, defensins also possess a number of other biological activities (chemotactic, mitogenic, cytotoxic). It has been revealed that defensins, depending on their local concentration, may exert opposite effects on cultured cells: in the micromolar concentration range they can exert mitogenic effects, whereas at higher concentra-

tions defensins are usually cytotoxic and cause lysis of tumor cells or may induce cell death via mitochondrial injury. It has recently been shown that some human tumors are characterized by an overexpression or down-regulation of certain defensin genes. The existing hypothesis on the functional role of defensins in tumorigenesis, and different approaches and experimental models used for the analysis of pluripotent activities of beta-defensin-2 in transformed human epithelial cells were presented.

Hypoxia, inflammation, and macrophages.

Antonio Sica (Fondazione per la Ricerca Humanitas, Milan, Italy) discussed a major leukocyte population mainly found in the hypoxic areas of tumors, the so-called tumor-associated macrophages (TAM), that are the principal component of the leukocyte infiltrate supporting tumor growth. Evidence is accumulating for a “switch” in macrophage phenotypes in the course of tumor progression. Whereas the functions of classically activated, “M1” macrophages in chronic inflammation appear to predispose a given tissue to tumor initiation, in established tumors, macrophages exhibit mainly the alternatively activated, “M2” phenotype and are engaged in immunosuppression and the promotion of tumor angiogenesis and metastasis. The regulatory mechanisms driving the functional plasticity of macrophages in the course of tumor development, along with their implication for anti-cancer therapies aimed at prompting TAM to mount an effective antitumor response were discussed.

Seth Coffelt (Sheffield University, UK) outlined the role of Tie2⁺ monocytes in tumor progression and response to therapy, and in particular the regulation by hypoxia and angiopoietin-2. It is known that tumor-associated macrophages (TAMs) actively contribute to tumor progression through stimulation of tumor cell proliferation, survival and metastasis, tumor angiogenesis, as well as suppression of the anti-tumor functions of other immune effectors at the tumor site. TAMs accumulate in hypoxic, peri-necrotic areas of tumors where pro-tumor activities of TAMs are promoted. This response to low oxygen tension is mediated by hypoxia-inducible transcription factors (HIF)-1 α and -2 α . The author and his colleagues have performed genome-wide microarray analyses of primary human macrophages exposed to hypoxia and found that more than 250 transcripts are regulated by hypoxia. Using an siRNA approach, specific knockdown of HIF-1 α and HIF-2 α in hypoxia-treated macrophages indicated that the subunits differentially regulate a broad array of genes and compensate for each other. Some authors have shown that tumor hypoxia also influences TAMs indirectly by stimulating the release of the pro-angiogenic cytokine angiopoietin-2 (Ang-2) from both tumor and stromal cells. Recent data from Dr. C. Lewis' laboratory and others implicate a subpopulation of TAMs expressing the Ang-2 receptor, Tie2 — termed Tie2-expressing monocytes (TEMs) — as being largely responsible for driving angiogenesis in tumors. Dr. Coffelt reported that Ang-2 stimulates the migration of TEMs and suppresses

their release of anti-angiogenic cytokines, whereas hypoxia increases expression of pro-angiogenic genes in these cells. It was concluded that a number of potent signals in the tumor microenvironment activate the tumor-promoting functions of TAMs and TEMs and that these cells are an important target for new anti-cancer therapies or combined therapies using anti-TAM agents to increase the efficacy of the conventional forms of treatment such as chemotherapy.

Lido Callorini (Florence University, Italy) demonstrated that tumor cells display a mesenchymal or an amoeboid type of migration when stimulated by humoral and cellular host factors and undergo an epigenetic change, respectively. Invasiveness through Matrigel was enhanced in B16 melanoma cells exposed to a medium conditioned by inflammatory macrophages co-cultivated with melanoma cells (tumor associated macrophages, TAMs). TAMs isolated from these co-cultures were found to express higher levels of MMP-9 compared to macrophage cultures alone. The pro-invasive activity of the TAM-conditioned medium was abrogated by anti-MMP-9 monoclonal antibodies. The results obtained indicated that B16 melanoma cells, following stimulation with an inflammatory cytokine or during the re-direction of protease expression in TAMs, express an increased invasiveness of mesenchymal type. It was also shown that the Ephrin tyrosine kinase A2 re-expression in B16 melanoma cells activates a non-proteolytic invasive program which proceeds through the activation of cytoskeleton motility, the retraction of cell protrusions, a Rho-mediated rounding of the cell body, and squeezing within the 3D matrix. These data suggest that EphA2 re-expression promotes an amoeboid-like migration in B16 melanoma cells. It was concluded that murine melanoma cells can shift from a mesenchymal to an amoeboid style of migration as an adaptation response, known as “plasticity”. It was also suggested that during the natural course of tumor progression, changes in protein expression and function — such as mutations in proteases or receptor tyrosine kinases — can alter the cellular phenotype of invasiveness.

Jörg Kleeff (Technical University, Munich, Germany) presented novel aspects of molecular biology in relation to pancreatic cancer. It is well known that pancreatic ductal adenocarcinoma (PDAC) is still a devastating, and in most cases, incurable disease. Current results support the concept that the stepwise progression of epithelial precursor lesions leads to invasive PDAC as a result of accumulating mutations in *K-ras*, *INK4A/ARF*, *TP53* and *DPC4*. In addition, a large number of growth factors and their receptors are up-regulated by epigenetic alterations in PDAC, and influence different aspects of the tumor pathophysiology. Established PDAC exhibits all the classic hallmarks of cancer, including self-sufficiency in growth signals, insensitivity to anti-growth signals, evasion of apoptosis, unlimited replicative potential, sustained angiogenesis, tissue invasion, and metastasis. Moreover, recent advances have identified essential elements of key pathways

partly recapitulating developmental signals and of the tumor microenvironment which promote tumor growth through the complex interplay of its different cellular components. The cancer microenvironment consists of various components including fibroblasts, endothelial cells, immune cells, and endocrine cells that interact with each other and the cancer cells in a complex fashion. Evidence is accumulating suggesting that the cancer microenvironment plays an active role in disease progression, and efforts are being made to target this interplay between cancer cells and host cells in order to improve the outcome of this deadly disease. For example, one promising new development is the appreciation of the role of the cancer-associated stroma. Current evidence supports the concept that cancer cells manipulate the stroma by co-opting their normal neighbors. Accordingly, stromal cells may depart from being normal, co-evolving with their malignant neighbors in order to sustain the growth of the latter. In this context, it has recently been shown that the activated stroma index is an independent prognostic marker in PDAC, highlighting the impact of the microenvironment on cancer progression and patient survival.

Hypoxia and invasion. *Claire Dubois (Sherbrook University, Canada)* focused attention on the involvement of LPA₁₋₃ signaling in invadopodia formation. It was shown that hypoxia increases the formation of the invasive structures, invadopodia, which degrade the extracellular matrix. It is known that autotaxin (ATX), which is upregulated in many malignancies, is a potent stimulator of cancer cell invasion. ATX possesses lysophospholipase D activity producing the bioactive lipid LPA which, signaling via G-protein coupled receptors LPA₁₋₅, influences cytoskeletal reorganization and matrix metalloproteinase activation that are essential components of invadopodia formation. Incubation of cancer cells under hypoxic conditions has been seen to result in an increase in the autotaxin gene and protein expression. Overexpression of autotaxin in HT1080 cells has also been demonstrated to result in an increase in the percent of cells degrading the matrix, the number of invadopodia per cell as well as the degradation area of the invadopodia. Using an antagonist of LPA₁₋₃ and specific agonists of LPA₂ and LPA₃, the author and her colleagues have defined the role of LPA/LPA₁₋₃ in invadopodia formation. It was also shown that Rock and Src are implicated in autotoxin-induced invadopodia formation. The results obtained have revealed hypoxia to be a novel inducer of autotaxin expression and suggest mechanisms by which hypoxia augments the invasion of tumor cells.

Dominique Arsenault (Sherbrook University, Canada) discussed aspects of the tumor invasion and metastasis triggered by the hypoxic microenvironment in tumors which involves a strategic relocalization of convertases, adhesion molecules, and metalloproteases. The highly invasive human fibrosarcoma cells HT-1080, transfected stably with eGFP-tagged-furin were used to study the impact of hypoxia on the cellular localization of furin. Hypoxia was shown to influence

the cellular relocalization of furin to the plasma membrane to two distinct regions: to focal adhesion points, characterized by a large concentration of integrins, and to sites of invadopodia, where metalloproteases were found. It was also demonstrated that casein-kinase II is implicated in the trafficking of furin and that filamin-A is essential for the membrane localization of furin under hypoxia. It was concluded that the observed relocalization allows furin access to different substrates such as integrins and metalloproteases, which are potentially implicated in the invasive and metastatic phenotype of tumor cells.

Reset Demir (Erlangen University, Germany) presented a unique *in vivo* model that has been developed to promote an understanding of a number of molecular pathways and cellular mechanisms for tumor invasion under hypoxia. For this purpose, fertilized chicken eggs were incubated for ten days under normoxic conditions. Subsequently, colon carcinoma cells (SW-480) were placed on the chorioallantoic membrane. During the following six days, the eggs were incubated either under normoxia or under stepwise decreasing hypoxic conditions. It was shown that SW-480 cells did not invade the epithelial layer under normoxic conditions. At the same time an invasion through the epithelial layer into the mesoderm has been already seen three days after commencement of incubation under hypoxic conditions. The chorioallantoic membrane assay presented by the author allows the investigation of tumor invasion and its mechanisms under defined hypoxic conditions.

Ludmila Sidorik (Inst. Mol. Biol. Genetics, NASU, Kyiv, Ukraine) presented data on molecular chaperones in cancer and discussed the question of whether they are the therapeutic targets or the therapeutic agents. The main attention was focused on heat shock proteins (HSP) that interact with multiple key components of signaling pathways regulating growth and development, survival and cell death. They are inducible in various intracellular pathways and overexpressed in a wide range of human cancers. They have been implicated as being involved in regulating tumor cell proliferation, differentiation, invasion, metastasis, recognition by the immune system and tumor cell death. The elevated HSP expression in malignant cells plays a key role in protection from spontaneous apoptosis associated with malignancy as well as the apoptosis induced by therapy. Strategies for the successful development of new anti-cancer therapies were proposed, including the pharmacological modification of HSP expression or molecular chaperone activity, and the use of HSPs as adjuvants for the presentation of tumor antigens to the immune system.

Svetlana Sidorenko (IEPOR, Kyiv, Ukraine) presented data concerning kinases of the PKD family as a potential object of translational research in oncology. It is well known that protein kinases are of special interest in cancer research due to their enzymatic activity and susceptibility to successful therapeutic targeting. The protein kinase D (PKD) family, which belongs to the CAMK group, comprises three closely related serine/threonine

kinases: PKD1 (PKCm), PKD2 and PKD3 (PKCn). This family of kinases occupies a unique position in the signal transduction pathways initiated by diacylglycerol (DAG) and PKC. Several lines of evidence suggest that PKD family kinases could be an object of translational research in oncology. PKDs are implicated in a wide array of cellular processes, including signal transduction, cell migration and survival, differentiation and proliferation, and there is a substantial amount of evidence linking PKDs to tumorigenesis. PKD1 has been found to play a direct role in the proliferation of endothelial cells, keratinocytes, T lymphocytes and pancreatic cancer cells. PKD1 may also play an anti-apoptotic role in cells and was also shown to be involved in the regulation of cell adhesion and motility. PKD1 interacts with components of the cadherin-catenin protein complex that are known to be involved in regulation of cellular aggregation and adhesion of cancer cells. Moreover, E-cadherin phosphorylation by PKD1 is associated with increased cellular aggregation and decreased cellular motility in prostate cancer. PKD1 is also found to be associated with cortactin and paxillin in invadopodia of breast cancer cells, indicating a role of this kinase in cancer cell invasion. The studies performed by the author and her colleagues are focused on PKD expression and activity in normal and malignant tissues of lymphoid origin, gastric and breast cancer cells. The correlation between the level of PKD expression and their activity and the stage of cell differentiation and activation status was described. It was emphasized that these kinases are novel proteins of interest with emerging translational value, especially in oncology.

Denis Kolesnik (IEPOR, Kyiv, Ukraine) presented data concerning interactions of endothelial cells and Lewis lung carcinoma cells with different angiogenic and metastatic potential. The interaction between endothelial and cancer cells not only promotes the vascularization of malignant tumors, but may also significantly affect their growth and metastasis. The work was aimed at studying the cross-interaction between endothelial cells (EC) and Lewis lung carcinoma cells (LLC) with different angiogenic and metastatic potentials (the strongly metastatic parental LLC with low angiogenic potential and its weakly metastatic LLC/R9 counterpart with high angiogenic potential) co-cultured *in vitro* under “contact” and “contactless” conditions. It was shown that “contact” co-culturing of exponentially growing murine aorta EC (MAEC) and LLC for 24 h results in significant inhibition of EC growth and stimulation of cancer cell proliferation. Interestingly, after 24 h of “contactless” co-culturing, LLC cell growth is inhibited without any changes in the number of MAEC. “Contact” co-culturing of exponentially growing MAEC and LLC/R9 cells does not lead to changes in the number of EC, whereas the inhibition of LLC/R9 growth was observed. 48 h “contactless” co-culturing of MAEC with LLC or LLC/R9 cells results in considerable growth activation of confluent EC cells. It was concluded that the differences in metastatic ability and angiogenic

potential of cancer cells are reflected in the nature of their interplay with endothelial cells.

Yurij Kudryavets (IEPOR, Kyiv, Ukraine) presented data on the influence of interferon- α (IFN) on levels of VEGF in the blood serum of breast cancer patients during combined therapy as well as IFN impact on disease outcome. It is known that IFN is one of the natural inhibitors of angiogenesis closely associated with tumor hypoxia. The use of IFN- α 2b in the combined therapy of breast cancer patients results in a decreased rate of disease progression over 36 months. VEGF levels in serum have also been shown to be significantly elevated after radiotherapy and surgery. Prolonged therapy with IFN in neoadjuvant and adjuvant regimens was accompanied by a significant decrease in the level of VEGF in the blood serum of patients. It was shown that in cases of repeated surgical treatment, VEGF levels increased significantly, as was observed after primary treatment. Combined therapy with IFN significantly inhibited the VEGF levels after repeated surgical treatment and decreased the relapse rate in these patients by more than two-fold. *In vitro* experiments have shown that under normoxic conditions, human cancer cells do not express HIF-1 α , and IFN- α treatment of cells failed to induce expression of HIF-1 α .

Nathalia Bezdenezhnych (IEPOR, Kyiv, Ukraine) presented data on the effects of long-term exposition of non-small cell lung cancer cells (A-549) to interferon (IFN) *in vitro*. It is known that IFN possesses pluripotential activity against tumor cells: it inhibits their proliferation and mobility, enhances apoptosis, modifies expression of surface receptors and antigens, suppresses angiogenesis in tumor, etc. IFN therapy is a part of the combined therapy of cancer patients that may continue for many months. Hence, it is of utmost importance to assess the effects of long-term exposure of cancer cells to IFN *in vitro*. A-549 cells were exposed to IFN at a range of increasing concentrations (100–10,000 IU/ml) over extended periods of time (22–360 days). Exposure to IFN resulted in the formation of cell resistance to the antiproliferative activity of the cytokine with the reversion of the malignant phenotype of cells (IFN-mod): the proliferative activity of IFN-mod cells was significantly reduced and the doubling time of the cells increased by a factor of 1.7 and was accompanied by a decrease in the expression of cell proliferation markers. The expression of E-cadherin (suppressor of invasion and metastasis) and the EGF receptor in IFN-mod cells increased and decreased, respectively. VEGF production by IFN-mod cells was also shown to be significantly and non-reversibly inhibited, as was VEGF expression in the cytoplasm. IFN-mod cells were seen to differ from the original ones by an increase in the number of cells with chromosomal rearrangement der(6)t(6;1) and a significant decrease of those with der(2)t(2;1) rearrangement. An increase of IFN concentration in the culture medium up to 10,000 IU/ml enhanced above-mentioned effects, but was accompanied by a simultaneous increase in toxicity. It was concluded that the

in vitro long-term exposure of A-549 cells to IFN results in the normalization of some malignant features of human lung cancer cells.

In conclusion, the general discussion emphasized the ominous impact of tumor hypoxia on aggressiveness and the progression of neoplasia, highlighting the possible clinical relevance of classifying human tumors according to their level of oxygenation. This may also be of relevance in the selection of the most efficient method of the treatment. A better understanding of the biology of malignant tumors and the development of effective anticancer modalities may be achieved

by studying features of the metabolic and stromal microenvironment of tumor cells which may be decisive in the outcome of tumor-host interactions.

ACKNOWLEDGEMENTS

The meeting was supported by NAS of Ukraine, sponsored by Charitable Foundation “Unity against Cancer”, Global Biomarketing Group, Nikomed, Biopharma, Orange, HVD, Hoffmann-La Roch and endorsed by European Society for Hyperthermic Oncology (ESHO). Authors thank Dr. Debra Kelleher for her editorial assistance.