Abridgement of the Ph.D. Thesis

The Relationship between Environmental Loads and Pathomechanisms

Dr. Regina Pálföldi

Instructors:

Dr. habil. Márta Gálfi
College Professor and Head of Department

Dr. habil. Attila Somfay
Professor and Head of Department

University of Szeged
Faculty of Science and Informatics
Doctoral School of Environmental Sciences

Szeged
2015

ABBREVIATIONS

ACTH	Adrenocorticotropic Hormone
Adh	Adenohypophysis
BCL2	B-cell lymphoma 2
ClB	Chlorobenzenes
EDC	Endocrine Disrupting Chemicals
MDR1	Multi drug resistance 1
PEHE	Pulmonary Epitheliod Haemangioendothelioma
PEHE M	Pulmonary Epitheloid Haemangioendothelioma – mixed cell culture
PEHE Tu	Pulmonary Epitheloid Haemangioendothelioma – tumorous cell culture
POP	Persistent Organic Pollutants
PRL	Prolactin
PRLoma	Prolactinoma
RIA	Radio Immuno Assay
TAX	Paclitaxel/Taxol
TX	Taxane
TXT	Docetaxel

INTRODUCTION

Homeostasis is the complex balance of living systems, which insures that the life phenomena of a given biological system take place in a continuous and optimized manner. In order to insure the stability of the internal environment, living systems configure adaptation patterns that may be described by process networks, thus they adapt to the constantly changing external environment as optimizing systems by creating a new balance.

The permanent and characteristic changes of the anthropogenic environmental conditions generate changes in biological systems in an exposition dependent fashion. The environmental load of even a sub-toxic dose of physical (electromagnetic radiation, ionizing radiation) and/or chemical (endocrine disrupting environmental chemicals /EDC/, or substrates used to model these substances, *eg* taxanes, chlorobenzene) agents may generate an altered environmental potential.

Environmental processes force homeostatic systems to create a chain of reactions; the first identifiable form of these is the modification and/or change of the behavior pattern, depending on the complexity of the individual. In this context behavior patterns are also crucial elements of the environmental adaptation potential. Behavior is the physiological consequence of the homeostatic processes of an organism characterized by species and individual, where the environment directly induces changes in the given biological system pattern (*eg* different body posture, movements). The expressed external and internal physical, instinctive and/or learned behavior patterns are characterized by the species and individual, in which activity or lack thereof, anxiety, fear, desire, aptitudes, emotions, the (quality of the) relationship to partners: aggression and dominance are determined.

In addition to behavior patterns, every creature adapts to the (physical, chemical and biological) changes of the environmental conditions by numerous well organized physiological processes. Environmental loads are linked to human societies. For this reason the research and exploration of the altered environment and the response-generating biological system is of utmost importance.

The social behavior (*eg* aggression, anxiety) of humans and the animal world may be herded from the healthy, homeostatic balance range to the socially unfavorably adapting, negative range by the changes in behavior pattern, caused by environmental loads (chemicals, radiation). As a consequence, the acquisition and utilization of the sources necessary for keeping internal balance may be hampered, which causes a shift in physiological response mechanisms. These

may lead to diseases via fixating negative physiological events in human and veterinary medicine. The described general diseases can be characterized by deviations even at a cellular level.

Environmental conditions are essential in healthy physiological functioning and the cell cycle, *ie* in the creation and maintenance of adaptation patterns of cells. These process systems may develop into inflammatory and transformational pathomechanisms as a result of environmental load. The different mediators released during inflammation may generate cascade events (*eg* development of metastases) when they reach the threshold value. Should these inflammations become chronic, they may turn into cell transformations, for example.

The treatment of tumors which developed as a result of the environmental adaptation potential loss in the biological system and/or its environmental conditions concentrates on the destruction of tumor cells (surgery, radiation therapy, chemotherapy). However, it does not take the possible environmental factors, which may be the cause, into consideration. Tumors (*eg* lung cancer) represent an enormous problem for society; they are actually mutation of the cell transformation process, and belong to the group of cell physiological disorders.

Most significant of the root causes are those which are capable of inducing cell transformational dysfunctions by creating a disturbance in the inner homeostatic balance (chronic expositions). EDC have such effects; they are present in our environment in natural (*eg* taxanes) and synthetic (*eg* chlorobenzenes) forms, and influence the homeostatic state of our organism. The fact that the above mentioned natural substances are suitable for modelling environmental load processes, is imperative from an environmental research point of view. It is known about some of these substances that they are able to inhibit cell proliferation, thus possess an antitumor effect. One such substance with a cytotoxic effect is taxane, synthesized by *Taxus brevifolia*, extracted and purified from the bark of the plant. Taxanes are natural environmental chemicals, which, in addition to the their general cell effects, change the regulation of the cell cycle, thus modifying the environmental adaptation potential of the biological system.

The 20^{th} century is the century of chemization in the history of our society. Many unnatural chemicals have been synthesized (persistant organic compounds /POP/), which bioaccumulate and are persistant, thus they have a wide spectrum of biological effects and possess toxicity characteristics.

Halogenated hydrocarbons are of particular significance, as many of them have an EDC effect. EDC attack the homeostatic balance of the endocrine system: the hormone, and/or target

receptor, and/or the functioning of the differentiated endocrine cells. Chlorobenzenes (ClB) are common artificial chemicals, which are suitable expositors in environmental load models.

Physical energy forms, which determine evolution, may come from natural or artificial (*eg* electromagnetic radiation) sources. Radiation effect is characterized by dose, its detection is well known in human medicine, as a part of treatment. Undifferentiated, young, and quickly proliferating cell populations are the most sensitive to rays. Ionizing rays force proliferating cells to take the apoptotic route (depending on dose and time period), thus enough accurate data is accessible on their effects on targeted, as well as non-targeted cells and tissues from therapeutic practice.

Physical (radiations) and/or chemical (EDC, POP) loads of the environment create numerous unbalanced adaptation patterns in living organisms, including humans. Medical science has only followed the unfavorable event sequences. As our country leads the cancer mortality statistics in the European Union (lung cancer has increased 10 fold in the last 50 years), the research of certain novel biological adaptation methods could be of particular importance. These may be such cell proliferation problems as new, rare tumorous diseases, *eg* PEHE (Pulmonary Epitheloid Haemangioendothelioma) may present the emergence of a real adaptation pattern, with the loss or modification of the current biological living substance pattern's adaptation potential. Since it is beneficial for the therapy to follow the environmental adaptation potential of PEHE disease, it is useful to find markers which are not only more effective in their therapeutic efficiency, but also bring us closer to the exploration of the general biological mechanisms. Signs which enable us to follow an adaptation pattern, freeze a given state at a given moment like a photograph, *eg* the positioning, size, cell specificity of a pathological anomaly. They do not supply information about the mechanisms, possible behavior, the epigenetic factors, the risks concerning the development of system algorithms of the new functional system. Therefore, it may be very important to know the dynamic data characterizing the behavior of the given dysfunctional processes.

With the loss of the environmental adaptation potential it is increasingly difficult to sustain the original active biological system states and the homeostatic processes. At the beginning of ontogenesis, the totipotent adaptable system has several physiological algorithms and heads toward new system balances. In many cases, these can be interpreted as diseases compared to the starting point. Chronic environmental effects cause changes which are hard to trace in the beginning. Later, as a result of permanent exposition, these systems use increasingly greater deviations (*eg* tumors) to signal the system's changes of state. It is an important fact that the system must be in balance not only with the external environment, but also with the internal, *eg*

intracellular milieu. It is questionable whether this internal environment, which is important for the biological sub-system (*eg* cell), can have such a marker role that makes us able to follow the state changes, which take place in the whole biological system, *eg* extracellular ionic milieu, mediators.

AIMS

In our research strategy we follow proven healthy states and their changes (transformations) via development of tumors in such a way that makes it possible to find characteristic markers for the state changes (alterations of trajectory) of the processes taking place, searching for the cause and effect relationship between condition modification and system changes. In the present work we wish to realize the examination of biological systems in such a way that in addition to the environmental potential determining external conditions, certain genetic and proteomic characteristics of the internal biological system algorithms are also followed.

Aim 1

We wished to interpret the effects (chemical: taxane, ClB; physical: ionizing radiation) which are important from an environmental viewpoint in a model, then characterize them according to their biological significance.

Aim 2

In our work it was our task to create and standardize adaquate research and investigation models (*in vivo* and *in vitro*) in addition to choosing exposition. The study of patient test results was considered highly important, due to their relevance in humans and the widening of the environmental science spectrum.

Aim 3

In the work with the created study models we also looked for the answer to the question of which could be the first relevant system responses of the psycho-neuro-endocrine system in the different EDC (ClB).

Aim 4

As a result of chronic exposition (*in vivo* sub-toxic - chemical and/or physical expositions), inflammation can develop and mediators are released. We wished to determine the possible role of these communication markers (*viz* mediators) in cell transformation.

Aim 5

We wished to find the answer to how could choosing the appropriate cancer treatment and/or the changes of the environmental potential be followed by new, dynamic methods.

Aim 6

Exploring the process directions responsible for sustaining the adaptation potential of the given biological system.

METHODS

In vivo studies

Medically certified male Wistar rats (bw: 180-350g) were used in our *in vivo* experiments. After conditioning, the animals were kept in standardized plastic cages, under controlled conditions. The smaller, unrelated, 4-6-week-old, 150-200g male Wistar rats were kept isolated in a different room from the start, under identical conditions. These were used as intruders in behavior studies. Tap water and laboratory rodent food was accessible *ad lib*.

The animals received 0.1 μg/bwkg and 1 μg/bwkg of a 1:1 mixture of 1,2,4-trichlorobenzene and hexachlorobenzene (ClB) in 1 mL of 0.015% aqueous ethanol daily via gastric tube. The dose and period of ClB exposition were created in accordance with data in the literature and our own needs. The ClB exposition of the rats lasted 30, 60 or 90 days. In our control system stress control, absolute control, negative and positive control groups were used. After the expositions, the following tests were done: open-field test (to determine behavior related to anxiety, locomotion and exploration) and resident-intruder test (to determine inter-male aggression in a neutral cage).

Bodyweight was measured after the last behavior test, decapitation was performed and blood samples were taken (to determine liver toxicity) under identical circumstances. Morpho-toxicological investigations were also done, signs referring to toxicity caused by ClB were monitored, and the general morphological examination was complemented by traditional histological staining of the sections from the given organs. Primary, monolayer cell cultures were made from the appropriate organs for further *in vitro* examinations.

The author wished to investigate the effects of non-target use of artificial ionizing radiation on the oesophagus in the patient group.

In vitro models

In our *in vitro* studies primary monolayer cell cultures were established, to investigate the living characteristics at sub-individual organization levels. The research models were made from the

organ samples of the above mentioned ClB treated animals, and from different cancerous human samples, *eg* lung, connective tissue, bone. The method for creating lung and lung tumor cell cultures was established employing the methods used for human and rat primary, monolayer cell cultures of hypophysis, glia cells and neurons, established earlier by our group. Explant cultures were made from another part of the tissue samples. Cell cultures were made from normal and cancerous tissues (PEHE), and we investigated the dose and time dependence of different chemical loads in three *in vitro* model systems: (I): intact tissue parts - low proliferation rate, and contact inhibition - this was the control; (II): tumorous clone - cells with space orientation disorder and loss of contact inhibition - PEHE Tu; (III): mixed: normal and cancerous cells - PEHE M.

Viability and incorporation studies were carried out and protein content of cell cultures was measured. Immunohistochemical experiments (BCL2; MDR1; CD34; CD31; Ki67, Factor VIII; estrogen, progesterone receptor) were performed on the tissue samples of the patient group and the cell cultures.

Chemical and physical expositors

Proven sub-toxic doses of the expositor ClB were used in both the *in vivo* and the *in vitro* animal experiments. In the study of the effects of taxanes, patient groups receiving taxane-based chemotherapy (paclitaxel: TAX 175 mg/m^2) were applied. The tissue samples which were treated with the appropriate sub-toxic doses in the *in vitro* studies also came from these patients.

For the physical exposition, the sub-toxic dose of artificial ionizing radiation was modeled by radio-chemotherapy protocols on the patient group (radiation dose: 25x1,8 Gy = 45 Gy + boost: 22-26 Gy, total dose: 67-71 Gy). The target was the macroscopic lung tumor.

Investigation of the changes in the ionic milieu

Cell culture models were made from the healthy and prolactinomic hypophyses (PRLoma) of female, 120-250 g, 4-6-week-old, medically certified Wistar rats. In the course of inducing a prolactinomic hypophysis, experimental animals kept under standardized conditions were given estron-acetate subcutaneously (150 μg/bwkg/week) for six months. As a result of the treatment, prolactinoma was detectable after six months in the hypophysis of the rats. In our experiments, only the [K^+] or the [Na^+] was modified, but the iso-states (ionia, hydria, osmosis, etc.) were kept. The hormone release of the primary cell cultures was studied in hypokalaemia

([K^+]: 0 mM; n=10) and hyponatraemia ([Na^+]: 0 mM; n= 8). Adh and PRLoma supernatant samples were collected on the basis of the experimental protocol set up according to the exposition dose effect and time; PRL and ACTH hormone contents were detected by RIA.

Investigation of the predictive nature of hyponatraemia in a patient group

Patients diagnosed with small-cell lung cancer between Jan 1, 2010 and July 1, 2014, at the Department of Pulmonology of the Csongrád County Chest Hospital formed our sample group, where incidence and severity of serum hyponatraemia, and its effect on survival were studied.

DISCUSSION IN LIGHT OF THE RESULTS

Permanent iteration takes place in the homeostatic process at the different organization levels of living systems, where the directed system elements (organ systems, organs, tissues, cells, intercellular spaces, etc.) are of complex functioning.

Iterating processes represent the follow-up of changes in circumstances, *id est* the visualization of the multitude of environmental changes from a process result point of view. Process ability is successful if the living individuals are able to adapt to their changing environment in the network of the given living environmental adaptation potential.

The more complete the environmental adaptation potential is, the more processes the living organism can apply to sustain the result (*eg* isothermia), if any of these is damaged, there still remain(s) compensational system element(s) which can insure that the organism can warrant its homeostasis in the changing environment.

It was our aim to plan, build and standardize models that are suitable for the follow-up of system dynamic changes (healthy - not significantly changed - inflammatory (several severity stages) - tumorous) created during exposition events that simulate the actual states. Creating such standardized investigation methods which make follow-up possible in sub-toxic doses, was regarded as an important research criterion.

In vitro (cell cultures) and *in vivo* systems were created for the study of environmental effects at sub-toxic doses. Special attention was necessary in the case of human studies for the choosing of patient groups in such a way that the sample analytical requirements are met for the statistical viewpoint.

Behavior studies were set up to investigate the effects of proven sub-toxic concentrations of ClB, which has an EDC effect at the level of the individual, on the psycho-neuro-endocrine system. Such a control system was created, where statistical analyses were carried out while

searching for deviations between the different control groups. It was determined that there are no significant, or even trend-like deviations between the control groups, therefore, only the results of the absolute control group (A /K/) were listed when interpreting further results. It could also be determined that as a result of chronic ClB treatment at a sub-toxic dose (0.1 and 1.0 μg/bwkg) behavior elements connected to aggression or anxiety intensified.

After the 90-day treatment every behavior element showed a significant deviation at both doses. However, the effects of the lower dose exposition did not present the described deviation even in the 60-day treatment period in every investigated behavior pattern. This result leads one to believe that a dose of 0.1 μg/bwkg ClB exposition approaches the effect threshold for aggressive behavior. Thus it can be said that the applied, proven sub-toxic ClB dose can trigger an unfavorable behavior pattern, which may further intensify the structurally (in its organism system) unfixed effect deviations caused by ClB. A further important finding is that the results gained by ClB expositions justify the reconsideration of social measures relating to the effects of halogenated hydrocarbons.

When artificial ionizing radiation is used under proven standardized and fixed circumstances in the course of radiation therapy, its apoptosis inducing effect is calculated for the target area. However, radiation also affects areas which are not targeted during therapy; this is when sub-toxic dose ionizing radiation can be detected. When targeted radiation therapy is used for the treatment of lung cancers, effects can also be found and followed in the untargeted esophagus; this way, in addition to insuring the triggering of inflammatory processes, cancer progression could also be followed. An association was found between the irregularities of the esophagus caused by radiation effect, and the dose-volume parameters.

If a structural biological system change (*eg* inflammation) brought on by a persistant (physical and/or chemical) environmental effect is proven, knowing which significant markers connected to its progression are worthy of investigation is a crucial question. Advanced stage chronic inflammatory processes often transform into a tumor, which signals the restructuring of the whole system behavior. In the present study we searched for those genomic markers which enable one to follow the described, rather long structure and the system cycle changing process. The quantitative follow-up of BCL2 and MDR1 expression - along with other markers, which are the subjects of our ongoing studies - makes it possible to study the structural disorders and the state cycle changes.

In addition to the chosen MDR1 and BCL2 general follow-up parameters, other specific markers, which help therapy, have also been determined. According to our *in vitro* immunohistochemical investigations on PEHE tumor, CD31: 3+; Ki67: +; CD34 and Factor

VIII loss was detectable, with estrogen and progesteron receptor negativity, and was coupled with a strong MDR1 protein presence (2+), and a low intracellular BCL2 level. In view of this, the effects of several cancer treatment agents (*eg* vinorelbine; carboplatin; docetaxel; paclitaxel) were studied in *in vitro* models for the sake of finding a definite therapy. The applied *in vitro* model study provided an opportunity to study dynamic cell behaviors on multigenerational cell cultures.

Promethazine, known from our earlier studies for its anti-plasmid effect, was also investigated in the present study. According to our results, the significantly decreased cell proliferation of PEHE cultures - in the presence of a chosen taxane (paclitaxel) - was further hindered with promethazine. The therapeutic scheme could be created accordingly, and was justified by its efficiency. The effects of the *in vivo* therapy were also followed by models created from newly acquired samples. The MDR1 inhibiting effect of promethazine was confirmed, as further *in vitro* treatment in the presence of docetaxel (TXT) significantly decreased cell proliferation. According to the present study, to follow *in vivo* tumorous states and to create a more efficiently targeted intervention, the application of dynamic *in vitro* models seems a valuable option. If tumorous processes are viewed as the narrowing of the spectrum of the environmental adaptation potential, then their behavior should be studied by the determining markers and process follow-up. When considering *in vitro* models, environmental conditions can be stabilized, this way the patient (biological sytem) can be supported after the potential remissions (or cures).

In the present thesis the dynamic co-operation of the environment and the system interpreted as a part of the environment, was followed by the changes of the system state cycle elements. Physical and chemical expositions were used as change provoking factors, with which modest disturbances (inflammations) were induced in the state cycle; as a result of their permanent presence structural disturbances were also detectable. Structural disturbances (*eg* those caused by chronic chemical expositions) can, naturally, be reversible or irreversible at the affected organization levels from the state cycle viewpoint. Since the medical database on this issue is significant, the inclusion of patient groups in our studies was warranted by the utilization and expansion of this database. At the same time, the fact that our results can be of benefit for health care, is important for our society as well.

Apart from increasing the effeciency of health care therapy protocols, it was also our objective to explore more accurately the prognosis of certain diseases (*eg* small-cell lung cancer). Our research data proved that extracellular hyponatraemia or hypokalaemia modifies the exocytotic activity of both healthy and tumorous cells. It also confirmed that the hormone secrection

kinetics of normal and cancerous cells shows variation, and decompensation is more marked in tumorous cell cultures in every case. In this context the cell, as a system, and its environment, as the condition of the system (*ie* its domain of attraction) collectively determine the current pattern, with which the cell sustains its complexity. Cells showing a modified state cycle exhibit a tumorous structure and process system.

The discrete shift of the extracellular ionic milieu can help determine the stage of the state cycle in the case of tumorous cells. According to our present knowledge, this only enables one to make prognoses; however, this, too, is significant for treatment. The protocols carried out *in vitro* examining the relationship between the local environment of cells and the cell functions, a strong association was found. We looked for hyponatraemia in cancer patients knowing this relationship, and if it was present, its suitability as a survival prediction marker was explored. As reported by our results, further consequent investigation of the ionic milieu in cancer patients is justified.

One purpose of our studies was the exploration of the environmental adaptation potential, which expression was introduced in the present thesis in order to make it possible to study the system and its domain of attraction as one unit. In this context we specified that the changes of the relationship between the system characterized by threshold effect and its environment is initially negligible, but cause increasingly greater process shifts. This phenomenon takes place because the system adapts, iterating its internal balance, to the changes of its environment in this way.

SUMMARY

New findings

1 Model systems suitable for the study of system associations defining *environmental adaptation potential* have been created.

2 The behavior (anxiety, aggression) disrupting effect of sub-toxic doses of a chemical environmental agent (ClB) has been confirmed. Furthermore, the significance of this effect from the perspective of the environmental adaptation potential has been shown.

3 The applicability of a follow-up method for minute disturbances caused by physical (artificial ionizing radiation in therapeutic doses: 45-71 Gy) exposition, coherently with the markers (BCL2, MDR1) of the structural disorder, has been confirmed.

4 Two methods supporting dynamic diagnosis and choice of therapy for PEHE have been created for the following of different state cycles. The dynamic behavior of tumor cells

showing a modified state cycle in tumor progression has been investigated from the signal marker (*eg* MDR1) point of view.

5 As a result of the modification (a decrease in the extracellular ionic concentration) of the environment or conditions (attraction domains) of tumorous and healthy cell cycles (attractors; Adh and PRLoma), they show differences in their complexity. The tumorous cultures are decompensated both in ACTH and PRL secretion.

6 Results gained by the investigation of the models of Adh and PRLoma attractors were extrapolated to human cases. Our hypothesis was confirmed according to the system biological approach: only the attraction domain extrapolation is possible at this time. Furthermore, it has been proven that the effects of changes of the isolated and small system elements (cell) can be compensated for, harmonized by the highly organized system (*eg* organism), but those of expansive and large system element changes cannot.

ACKNOWLEDGEMENTS

I would like to express my sincere appreciation to my instructors, Dr. Márta Gálfi, habilitated college professor and head of department, and Dr. Attila Somfay, professor and head of department, for their reliable help through the course of this work. I am indebted to the members of our research team: Dr. Marianna Radács, college associcate professor, Zsolt Molnár, college assistant lecturer, László Dezső Rácz, engineer, and Péter Miczák.

I would like to thank Dr. Anna Juhász and Dr. Zsuzsanna Valkusz for their valuable suggestions and pieces of advice.

I am especially thankful to Dr. Klára Szalontai, head physician and my colleagues at the hospital for their support, as well as to Dr. Beatrix Bálint director for her supportive attitude.

Finally, I thank my Family for their understanding, love and encouragement throughout the years.

I am thankful to TÁMOP4.1.1.C-12/1/KONV-2012-0012, TÁMOP-4.2.2.A-11/1/KONV-2012-0035 and the Hungarian Pulmonology Foundation.

PUBLICATIONS ON WHICH THE Ph.D. THESIS IS BASED

1 **R. Palfoldi,** M. Radacs, E. Csada, Z. Molnar, S. Pinter, L. Tiszlavicz, J. Molnar, Z. Valkusz, A. Somfay and G. Galfi, *Pulmonary epithelioid haemangioendothelioma studies in vitro and in vivo: new diagnostic and treatment methods.* In Vivo 27(2):221-5, (2013).

Impact factor: 1,148

2 A. Maráz, J. Furák, Z. Varga, E. Fodor, Z. Együd, E. Borzási, Z. Kahán, **R. Pálföldi**, L. Tiszlavicz and K. Hideghéty, *Acute oesophageal toxicity related to paclitaxel-based concurrent chemoradiotherapy for non-small cell lung cancer*. Anticancer Research 33(4):1737-1741, (2013).

Impact factor: 1,872

3 A. Maraz, J. Furak, **R. Palfoldi**, J. Eller, E. Szanto, Z. Kahan, L. Thurzo, J. Molnar, L. Tiszlavicz and K. Hideghety, *Roles of BCL-2 and MDR1 expression in the efficacy of paclitaxel-based lung cancer chemoradiation*. Anticancer Research 31(4):1431-1436, (2011).

Impact factor: 1,725

4 Z. Molnár, **R. Pálföldi**, A. László, M. Radács, M. László, P. Hausinger, L. Tiszlavicz, Z. Rázga, Z. Valkusz and M. Gálfi, *The effects of hypokalaemia on the hormone exocytosis in adenohypophysis and prolactinoma cell culture model systems.* Exp and Clin Endocrinol & Diabetes 122(10):575-81, (2014).

Impact factor: 1,76

5 **R. Palfoldi**, M. Radacs, E. Csada, Z. Rozsavolgyi, L. Tiszlavicz, A. Somfay and M. Galfi, *Primery pulmonary epitheloid haemangioendothelioma.* Orv hetilap 152(21):834-9, (2011).

6 Z. Valkusz, G. Nagyeri, M. Radacs, T. Ocsko, P. Hausinger, M. Laszlo, F. A. Laszlo, A. Juhasz, J. Julesz, **R. Palfoldi** and G. Galfi, *Further analysis of behaviorl and endocrine consequences of chronic exposure of male Wistar rats to subtoxic doses of endocrine disruptor chlorobenzenes.* Physiol Behavior 103(5):421-430, (2011).

Impact factor: 2,869

POSTER AND ORAL PRESENTATIONS RELATED TO THE Ph.D. THESIS

1 **Pálföldi R,** Radács M, Csada E, Rózsavölgyi Z, Tiszlavicz L, Valkusz Zs, Somfay A, Molnár J, Gálfi M, *Antitumor treatment of pulmonary epitheloid haemangioedothelioma – after in vitro tumor cell cultures experiments*. In: 22nd International Congress on Anti-cancer Treatment. Place and date of conference: Paris, France, 2011.02.01-2011.02.04. *Paper IC/AB1171.*

2 **Palfoldi R**, Radacs M, Csada E, Rozsavolgyi Z, Tiszlavicz L, Valkusz Z, Somfay A, Molnar J, Galfi M, *Antitumor treatment on pulmonary epitheloid hemangioendothelioma.* Journal of Thoracic Oncology 6 (6) *pp. S1523-S1524*, (2011).

3 **Palföldi R**, Csada E, Rózsavölgyi Z, Tiszlavicz L, Gálfi M, Somfay A, *Pulmonary epitheloid hemangioendothelioma,* In: Zsolt Pápai-Székely (szerk.), 16th World Congress for Broncholgy. Place and date of conference: Budapest, Hungary, 2010.06.13-2010.06.16.*Paper A-0169.*

4 **Pálföldi R**, Csada, E, Rózsavölgyi Z, Tiszlavicz L, Gálfi M, Somfay A, *Terápiás megoldások támogatása in vitro módszerek fejlesztésével.* Medicina Thoracalis (Budapest) 63**:** p. 217, (2010).

5 **Pálföldi Regina** et al.: *Pulmonalis epitheloid haemangioendothelioma*. In: Magyar Bronchológus Egyesület konferenciája. Place and date of conference: Pécs, Hungary, 2009.10.09-2009.10.10.

6 **Pálföldi R,** Szalontai K, Csada E, Furák J, Tiszlavicz L, Somfay A, *A hyponatraemia kissejtes tüdőtumoros betegeinkben.* In: A Magyar Tüdőgyógyász Társaság Onkolulmonológiai Szekció és a Magyar Bronchológus Egyesület konferenciája. Place and date of conference: Szeged, Hungary, 2014.10.09.-11.