

# Workshop



## OMICS FOR ASSESSING UNCLEAR RISKS

# Abstracts

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## SCOPE

Risk assessment with regard to carcinogenicity so far is largely based on evidence from epidemiological and animal studies while *in vitro* studies only play a supporting role. Recently, however it has been suggested that new developments in omics technologies (genomics, proteomics, metabolomics etc.) might change this profoundly. In particular, omics technologies might be used to investigate potential modes of action regarding carcinogenicity more effectively than current research based on genotoxic studies can do. Omics technologies may also play an important role in identifying susceptible populations and life-stages as well as in animal-to-human extrapolation.

The aim of the workshop is to explore the potential of omics and cytogenetic technologies for cancer risk assessment, especially with respect to radio-frequency electromagnetic fields (RF-EMF) exposure. The risk of exposure to RF-EMF is unclear because reproducible hazards are not known. It will be discussed how insufficient knowledge of possible hazards can be used in risk assessment, a pressing question in modern societies.

This workshop brings together international experts with different scientific backgrounds from omics and genotoxicity research, as well as from the field of RF EMF animal and epidemiological research. We expect that this will stimulate an open discussion about the pros and cons of omics for assessing unclear risks. The workshop is open to all interested parties and stakeholders.

### THE ORGANIZING COMMITTEE

Gerd Friedrich – Forschungsgemeinschaft Funk e.V.

Peter Nürnberg – Cologne Center for Genomics

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Peter Wiedemann - Jülich Research Centre"

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RF EMF CANCER RISK  
ASSESSMENT:  
WHAT DO WE KNOW,  
WHAT DO WE NEED TO KNOW?

# MODELS OF CANCER DEVELOPMENT: GENETIC AND ENVIRONMENTAL INFLUENCES

*TBA*

# **CYTOGENETIC STUDIES IN MAMMALIAN SOMATIC CELLS EXPOSED TO RADIOFREQUENCY RADIATION: PAST, PRESENT AND FUTURE**

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During the last several decades, several researchers have investigated the potential genotoxic effects of *in vitro* and *in vivo* exposure to radiofrequency radiation (RFR) in mammalian somatic cells. Vijayalaxmi and Obe (2004) have reviewed the data reported in a total of 53 peer-reviewed scientific publications during the years 1990-2003. The conclusions were: (i) the results from most of the studies (58%) did not indicate increased damage to the genetic material (assessed from DNA strand breaks, incidence of chromosomal aberrations, micronuclei and sister chromatid exchanges) in RFR-exposed cells as compared with those in sham exposed and/or un-exposed cells, (ii) some investigations (23%) have reported an increase in such damage in RFR-exposed cells, and (iii) the observations from other studies (19%) were inconclusive. The potential causes for the conflicting data were discussed in the review. Also, a recommendation has been made to conduct a well coordinated, multi-centered collaborative investigation with adequate statistical power to identify the factors that have contributed to these controversial observations.

At present, two independent laboratories in Germany are participating in such an investigation.

Future prospects for cytogenetic investigations in RFR research will be discussed.

## CONCLUSIONS FROM ANIMAL STUDIES

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Carcinogenesis (i.e. the development of cancer) is a complex, multi-stage process. Through the phases of initiation, promotion and progression after a latency of years to decades, the process of carcinogenesis leads from the initial transformation of normal cells to a clinically manifest tumour. For a sound risk assessment of potential causative factors, animal studies using lifetime-exposure are of highest priority. In previous reviews, the lack of such animal studies with electromagnetic fields (EMF) was identified as one of the major knowledge gaps in the process of carcinogenicity risk assessment. However, studies of this type formed part of the EU research program **PERFORM A**, which was recently finalized and the results presented in 2007.

This project consisted of four different subprojects, which included the investigation of the handset exposure from the dominant mobile communications systems in Europe, i.e., GSM (Global System for Mobile Communications) and DCS (Digital Personal Communications System) at three exposure levels (and sham) on rodents to allow the detection of a potential dose response relationship. The final report of the program is available at [www.item.fhg.de/PERFORMA.pdf](http://www.item.fhg.de/PERFORMA.pdf) and the results can be summarized as follows:

Under the conditions of study **PERFORM-A1**, the exposure of male and female B6C3F1 mice to electromagnetic fields (EMF) of GSM and DCS wireless communication signals at a whole body absorption rate of up to 4.0 W/kg, two hours per day, 5 days per week, over a period of up to 24 months produced no evidence that the exposure had any adverse health effect or any influence on the incidence, severity, or time of appearance of the background non-neoplastic and neoplastic lesions observed.

Under the conditions of study **PERFORM-A2**, Wistar rats exposed to 902 MHz GSM Wireless Communication Signals at SAR levels of up to 0.44, 1.33 or 4.0 W/kg or 1747 MHz DCS Wireless Communication Signals at SAR levels of 0.41, 1.23 or 3.7 W/kg for 2 hours/day and 5 days/week over a period of up to 24 months produced no evidence that the exposure had any adverse health effect or any influence on the incidence, severity, or time of appearance of the background non-neoplastic and neoplastic lesions observed.

Under the conditions of study **PERFORM-A3** it is concluded that this study produced a borderline evidence of long term repeated exposure to 902 MHz GSM signals affecting the DMBA-induced mammary tumour response in rats with an equivocal biological relevance.

Under the conditions of study **PERFORM-A4**, exposure of *Pim 1* transgenic mice to a pulsed 900 MHz electromagnetic field (EMF) at an absorption rate of 0.5, 1.4 or 4.0 W/kg, daily for one hour, over a period of not less than 18 months produced no evidence that exposure had any effect on the incidence or severity of any neoplastic or non-neoplastic condition.

As an overall conclusion, three out of four studies produced no evidence that exposure had any effect on the incidence or severity of any neoplastic or non-neoplastic condition. The only effect observed is a borderline one in the study investigating effects on the DMBA-induced mammary tumour response. With these new results in mind, the following points will be discussed and future steps will be suggested:

1. Can these results be extrapolated in the same way as it would be possible for chemicals ?
2. What is the contribution of dosimetry to improve this process of extrapolation ?
3. What is the potential role of "omics" studies in the process of risk assessment ?

# TUMOR PROMOTION BY CHRONIC UMTS-MODULATED RADIOFREQUENCY EXPOSURE IN MICE PRENATALLY TREATED WITH ENU

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**OBJECTIVE:** To evaluate possible influences on tumor development in freely moving mice following exposure to a generic UMTS (universal mobile telecommunications system) test signal for 20 hours/day on 7 days/week, starting as fetal exposure.

**METHODS:** The experiment was carried out as a one-generation study with prenatal exposure, using two EMF treatment groups, namely the "UMTS high-dose group" and the "UMTS mid-dose group" with additional ethylnitrosourea (ENU) treatment, and a sham-exposed control group in the EMF exposure device, in addition to an untreated cage control group and a positive (ENU-treated) control group. Starting on day 6 of pregnancy maternal mice were UMTS-exposed, while lifetime exposure (up to 24 months) of the F1 descendants started on the day of birth. Maternal ethylnitrosourea administration (40 mg ENU/kg b.w.) was carried out on day 14 of pregnancy. Each treatment group consisted of 20 maternal mice and their litter. After the first week (litter standardization), 3 female descendants and their mothers were maintained per cage. After weaning (week 3), maternal mice were removed from the cages, so that the long-term study was conducted using three female descendants per cage.

The exposure was performed in a three-level exposure unit [sham, medium-dose (4.8 W/m<sup>2</sup>), highdose (48 W/m<sup>2</sup>)] consisting of three stacked radial waveguides and housing up to 60 female B6C3F1 mice (3 per cage) per level. Histopathological examination by light microscopy was limited to the brain, lungs, liver, spleen, kidneys, mesenterial lymph nodes, and gross lesions. Neoplasms and pre-neoplastic lesions were diagnosed and classified according to the WHO/IARC nomenclature.

**RESULTS AND CONCLUSION:** Mortality in both ENU groups increased after 12 months lifetime confirming the prenatal/maternal ENU treatment. The remaining survivors were necropsied when mortality exceeded 75 % in both ENU groups (week 75). Cage controls, sham, and the UMTSexposed mice showed similar mortality rates up to terminal sacrifice (week 104). The cage control group, sham exposure group and UMTS high-dose group revealed comparable tumor incidences in the target organs. The UMTS high-dose group, in contrast, showed a significantly increased number of pre-neoplastic liver foci as compared to the sham control and cage control groups. Analysis of the neoplastic and pre-neoplastic findings in the two ENU groups revealed some remarkable findings:

- A comparison of neoplastic lesions revealed an increased liver tumor rate and a significantly increased lung tumor incidence in the ENU/UMTS group as compared to the ENU control group.
- Incidences of hepatocellular adenoma(s) and bronchiolo-alveolar carcinoma(s) were significantly increased in the ENU groups after lifetime UMTS exposure.
- With respect to bronchiolo-alveolar carcinoma(s) and hepatocellular adenoma(s), tumor multiplicity was significantly increased in the ENU/UMTS group as compared to the ENU control group.
- The incidence of metastasizing lung carcinoma(s) in the two ENU groups was doubled by the long-term UMTS exposure of the mice.
- In addition, the incidence of pre-neoplastic hepatocellular foci also increased significantly in the ENU/UMTS group as compared to the ENU control group.

In conclusion, the study revealed distinct tumor-promoting effects of chronic UMTS exposure in this ENU mouse model. It currently remains unclear, however, to what extent these limited results (promotion of ENU-induced tumorigenesis by long-term UMTS exposure) are predictive of human carcinogenesis. Further investigations are needed to verify the present findings and obtain reliable information regarding their relevance in humans.

Support of the project by Compagnia di San Paolo, Torino, Italy, is gratefully acknowledged.

## CONCLUSIONS FROM EPIDEMIOLOGICAL STUDIES

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The exposure of the population to radio frequency electromagnetic fields (RF-EMF) has been increased in recent years, especially since the introduction of mobile phones. Since then, adverse health effects have been a matter of controversial debates. But up to now there are no explicit, generally accepted results on cancer effects of RF-EMF, as far as the limit values are not exceeded. Animal studies have not provided evidence that RF-EMF could induce cancer. Up to now, there are no generally accepted mechanisms on how RF-EMF might affect cancer genesis. With regard to epidemiological data also no clear evidence can be seen. Firstly this is caused by the difficulties of a valid estimate of RF-EMF exposure on the job and in leisure time. Secondly, until now only few persons are exposed for a long time to RF-EMF like mobile communication. In particular the use of mobile phones and the incidence of brain tumor are discussed in detail. Even though, up to now it might only be concluded that an increased health risk during the first ten years of mobile phone use might be excluded. But up to now, there is still insufficient data to exclude an increased risk for long term use of mobile phones.

# ASSESSING HEALTH RISKS FROM RADIOFREQUENCY FIELDS: A WHO PERSPECTIVE

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WHO's assessment of any health risks produced by EMF emitting technologies falls within the responsibilities of the International EMF Project and the International Agency for Research on Cancer (IARC). A thorough investigation of the health impact of static and low frequency EMF has been performed by both the International EMF Project<sup>1,2</sup> and IARC<sup>3</sup>, and radiofrequency fields are due to be evaluated shortly.

The WHO health risk assessments are the result of in-depth critical reviews conducted through independent, scientific peer-review groups. They are usually undertaken if new data are available that would substantially change the evaluation, if there is public concern for health or environmental effects of the agent because of greater exposure, or if an appreciable time period has elapsed since the last evaluation.

These assessments are intended to provide critical reviews on the effect on human health of radiofrequency fields. As such, they will include and review studies that are of direct relevance for the evaluation. In the evaluation of human health risks, sound human data, whenever available, are generally more informative than animal data. Animal and in vitro studies provide support and are used mainly to supply evidence that is missing from human studies.

All studies, with either positive or negative effects, need to be evaluated and judged on their own merit, and then collectively evaluated and judged in a weight of evidence approach. Generally, studies must be replicated or be in agreement with similar studies. The evidence for an effect is further strengthened if the results from different types of studies (epidemiology or laboratory) point to the same conclusion.

These assessments are intended to assist national and international authorities in making their own risk evaluation and subsequent risk management decisions. They represent a thorough evaluation of risks and are not, in any sense, recommendations for regulation or standard setting. These latter are the exclusive purview of national and regional governments.

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<sup>1</sup> WHO - World Health Organization. Static Fields. Environmental Health Criteria, Vol. 232. Geneva, World Health Organization, 2006.

<sup>2</sup> WHO - World Health Organization. Extremely Low Frequency Fields. Environmental Health Criteria, Vol. 238. Geneva, World Health Organization, 2007.

<sup>3</sup> IARC Working Group on the Evaluation of Carcinogenic Risks to Humans. Non-ionizing radiation, Part 1: Static and extremely low-frequency (ELF) electric and magnetic fields. Lyon, IARC, 2002 (Monographs on the Evaluation of Carcinogenic Risks to Humans, 80).

# THE CONTRIBUTION OF GENOTOXIC RESEARCH TO RISK ASSESSMENT

## VALIDITY AND RELIABILITY OF GENOTOXIC STUDIES FOR CANCER RISK ASSESSMENT

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The close correlation between chromosomal aberrations (CA) and cancer makes studies of cytogenetic effects of environmental and occupational agents extremely important.

Chromosomes contain mainly DNA, histone and non-histone proteins. Before DNA-synthesis (G1 phase), each chromosome contains one continuous DNA molecule which is replicated in S-phase. After a short G2 phase chromosomes condense and are generally analyzed in mitotic metaphase in which chromosomes contain two chromatids with one DNA molecule each. After induction of premature chromosome condensation (PCC), CA can be analyzed in G1 and G2 phases. CA can also be analyzed in different stages of meiosis.

DNA is the target for the formation of CA by mis-repair of various types of DNA damage. Ultimate lesions for the formation of CA are DNA double-strand breaks (DSB) which when un-repaired would lead to loss of chromosome material containing plenty of genes and in consequence to cell death. DSB repair restores the original structure of DNA, but occasional mis-repair may lead to CA. Types of CA depend on the cell cycle stage in which DSB occurred. Mis-repair of DSB in the G1 phase give rise to chromosome type aberrations such as dicentric chromosomes or translocations. Cells of many cancers are characterized by specific translocations which have diagnostic value for the cancer in question. In G2-phase DSB lead to chromatid type CA and during S-phase to both types of CA, dependent of whether DSB occurred in still un-replicated or already replicated DNA. This is true for agents directly inducing DSB, such as ionizing radiation and endonucleases, but not for agents inducing single-strand lesions (SSL) such as base damage, cross-links or single-strand breaks. Most clastogenic agents induce SSL which when present in replicating DNA may give rise to DSB in one DNA strand and in consequence to chromatid type CA. Therefore, exposure of G1 cells to CA inducing agents and analysis of first post-treatment metaphases (M1) allows deciding of whether an agent induces DSB or SSL.

Since RF-EMF are not able to induce DSB, exposure of G1 cells can not lead to dicentric chromosomes or translocations in M1. If exposure to RF-EMF would produce SSL, chromatid-type aberrations should occur in M1. From the data available it rather looks as if RF-EMF are not directly inducing SSL. If this is true, CA could still occur by indirect mechanisms such as formation of radicals which could give rise to SSL. Evidences for indirect mechanisms following exposure to RF-EMF are rather scanty.

Chromosome and chromatid type CA can lead to micronuclei (MN) during anaphase. MN may contain centromere-less chromosomal fragments derived from CA, or whole chromosomes with centromeres which failed to be properly distributed in anaphase. These MN types can be differentiated by methodologies which allow visualizing centromeres inside MN.

Another test for SSL is based on the analysis of sister-chromatid exchanges (SCE) which mainly result from repair of SSL by homologous exchange events. SCE do not indicate that a test agent is mutagenic, but that it induced damage in DNA which was repaired in an error-free manner.

One popular test in RF-EMF research is the comet assay based on the finding that damaged DNA leaks out from cell nuclei by electrophoresis resulting in comet like structures. The comet assay is an indicator test for DNA damage, but does not proof that a test agent is mutagenic.

In order to get valid and reliable information about a mutagenic/carcinogenic activity of an agent, cytogenetic tests should be done following the rules of Good Laboratory Practice (GLP). In the frame of cytogenetic analyses, the most direct information about a mutagenic activity of an agent is obtained by the CA test. An agent positive in the CA test would induce centromere-free MN. An agent negative in the CA test could induce MN with centromeres. If chromosome type CA are induced following exposure of cells in G1, the agent in question would be negative in the SCE test. A positive SCE test indicates that the agent applied induces SSL, the CA test would show chromatid type CA in this case. The comet assay should be positive when CA, SCE and centromere-free MN are induced, but negative when only centromere-containing MN occur.

In order to avoid false positive or false negative results concerning mutagenic/carcinogenic effects of a given agent, it is recommended to use a test battery which in addition to tests of cytogenetic effects in vitro and in vivo should also include tests for gene mutations in bacteria and mammalian cells.

## **CHROMOSOMAL ABERRATION ANALYSIS. IS IT INFORMATIVE FOR RF-EMF EXPOSURE?**

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Chromosomal aberration analysis, using human cells, is a long standing (~50y) field of research that now occupies an important place in mutagen studies. This is because many genotoxic agents, chemical and physical, in man's environment can be shown to cause damage visible at the chromosomal level of organisation. Cytogenetic observations with such 'clastogenic' agents can usually be linked to plausible mechanisms of their action and are thus amenable to reproducible experimental variations in vitro and sometimes in vivo. To date, there is no obvious mechanism that can link RF exposures, at non-thermal levels, to the breakage of DNA or disruption of its repair mechanisms that eventually lead to visible chromosomal aberrations.

Nevertheless, there have been numerous cytogenetic studies undertaken with EMF, at both RF and ELF, and some, albeit a minority, do report positive effects. These have been extensively reviewed by Drs Vijayalaxmi and Obe. For a variety of reasons the human lymphocyte has been the most frequently used cell for these studies.

A thread seems to run through the literature of failure to reproduce the reports of positive effects when they are investigated in other laboratories. Thus findings of both direct clastogenesis, and also epigenetic effects, whereby RF potentiates the effect of another well-established mutagenic agent do not hold up, and in many instances the reasons remain unresolved. Similarly, suggestions that the mainstay cell, the lymphocyte, is an inappropriate assay tool because effects can only be demonstrated in other cell types have proved elusive.

These conundrums will be illustrated with a few examples. A widely held view in the cytogenetics fraternity is that RF-EMF is not demonstrably genotoxic and unless a plausible mechanism for a clastogenic effect emerges it may be time to halt such work. A lot of time, effort and money has been expended, often fuelled by the public's perception of risk. Of course, it is appreciated that genotoxicity may occur in other ways, without expression at the chromosomal level, but a message to the omics fraternity is that you could learn from this experience.

## DOING RISK ASSESSMENT BASED ON GENETIC DATA

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Environmental stressors, including radiation, elicit a number of detectable changes at the molecular, cellular, and physiological level in exposed organisms. These biological parameters have been called *biomarkers* [1]. Since a biomarker can provide different outputs that relate to different endpoints, we can divide them into *dose indicators* or *risk indicators*. A biodosimeter is a biomarker of exposure, i.e. a biological parameter that is sensitive to the energy released by radiation. Sensitive dose bioindicators are essential when no physical dosimetry is available, e.g. in nuclear accidents. For instance, electron spin resonance (ESR) in tooth enamel is recognized as a highly sensitive biodosimeter that can be used to measure dose many years after exposure has occurred (retrospective biodosimeter). However, ESR is a measure of the absorbed dose, not of the *biologically relevant dose*. Biologically relevant dose represents residual damage following repair, is dependent on genetic predisposition, diet, stress, etc., and is correlated to the late effects. Chromosomal aberrations (CA), for example, reflect biologically relevant dose and represent a risk indicator. In fact, recent epidemiological studies have demonstrated that CA in blood lymphocytes of healthy individuals are predictive of cancer risk [2]. The presence of CA in the blood lymphocytes suggests genomic instability, and may reflect similar instability processes in the target organs. An interesting application of CA as biomarker is in astronauts involved in long-term space flights, where the uncertainty on risk are so high that biomarkers greatly help to validate the models [3]. The best method of assessing CA, and timing between exposure and testing remain open issues. Novel molecular methodologies based on genomics and proteomics show promise as screening and clinical diagnostic tools at low-doses, but they require further development and validation studies. It has been suggested that an individual's response using a validated biomarker should be part of the medical record [4]. However, so far only CA can be considered as a validated biomarker, and it will take many years for any other parameter to undergo time-consuming epidemiological studies.

### REFERENCES

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# COMPARATIVE RISK ASSESSMENT WITH IONIZING AND NON-IONIZING RADIATION

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Risk assessment of ionising radiation is based on an established system of available data. The most important source are epidemiological studies, mainly but not exclusively on the survivors of atomic bomb explosions in Hiroshima and Nagasaki. These data are supplemented by numerous investigations in animal populations and experiments on cells and molecules. Hazards identified are both of deterministic and stochastic nature, characterised by threshold type and non-threshold type dose responses, respectively. Based on this information dose limits can be established with some reliability. With ultraviolet rays (UV) there is some information on carcinogenic action in humans based on ecological epidemiological studies. Animal investigations provide clear evidence for the carcinogenic action of UV. Initial processes appear to be well understood from laboratory experiments. Deterministic effects, e. g. erythema, show exposure thresholds which are well known from human experience. The dose dependence for UV-induced skin cancers in men is not well established and has large uncertainties. Radiofrequency electromagnetic waves are known to lead to thermal effects in tissues which are deterministic in nature. Exposure thresholds estimates are based mainly on experiments in model systems and calculations. There is no epidemiological or experimental evidence for non-stochastic effects of radiofrequency electromagnetic fields.

The different protection systems are discussed and the available evidence for hazard assessment as well as the limitations is outlined.

## IMPORTANCE OF EPIGENETIC CHANGES IN CANCER

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The term “epigenetic” refers all heritable changes in gene expression and associated phenotypic traits that are not coded in the DNA sequence itself, and it is now recognized that epigenetic mechanisms are critical for understanding the causes of complex diseases such as cancer. The potential utility of epigenetics in cancer research is highlighted by the fact that many funding agencies put cancer epigenetics on the priority list. Epigenetic events play key roles in the control of key cellular process and their deregulation has been associated with virtually all stages of cancer development and progression. A number of critical processes found in cancer cells, such as silencing of tumour suppressor genes, activation of oncogenes, aberrant cell cycle, and defects in DNA repair, can be caused by aberrant epigenetic states. Epigenetic inheritance include DNA methylation, histone modifications, and microRNAs, all of which are essential mechanisms that allow the stable propagation of gene activity states from one generation of cells to the next. Although the role of epigenetic events is supported by both epidemiological and experimental studies, the precise contribution of epigenetic mechanisms and cellular targets epigenetic alterations to human cancers are largely unknown. Almost spectacular technological advances in epigenetics and epigenomics now allow powerful screening of large series of samples with unprecedented resolution. These approaches are beginning to reveal a number of genes (tumor suppressors and other cancer-associated genes) susceptible to inactivation through epigenetic mechanism. Epigenetic profiling using both genome-wide and candidate-gene approaches in normal tissues and different tumor types will help to elucidate the mechanism underlying tumourigenesis. Technological advances in epigenetics and epigenomics have now been exploited to identify specific epigenetic targets, environmental factors, and the critical windows of vulnerability to environmentally induced epigenetic changes in cancer. A list of genes and gene networks that are targets of epigenetic alterations are likely to grow with the progress of major programmes in the field. Recent technological advances in epigenetics and epigenomics and new emerging concepts involving epigenetic events as well as their implication for mechanistic understanding of carcinogenesis, cancer risk assessment and prevention will be discussed.

## **VALIDITY AND RELIABILITY OF CURRENT OMICS STUDIES FOR CANCER RISK ASSESSMENT**

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One of the most widely used tool in omics studies is the microarray, which enables the determination of the steady-state levels of mRNA of almost all known genes of the genome. By analyzing control samples and samples exposed to suspected toxic compounds by microarray techniques, a possible effect of the compound on gene expression can be investigated. By analyzing the resulting gene expression changes, characteristic gene signatures or patterns indicating exposure, initiation of toxic events and mechanism, as well as carcinogenic potential might be identified.

During the past five years, a number of studies have made use of microarrays to investigate effects of RF EMF exposure on gene expression. As part of the project "Implications of Biomedicine for the Assessment of Human Health Risks (IMBA)", experimental design, techniques, and analysis strategies of these studies have been evaluated in detail. Overall, this study revealed a wide variety of samples types, microarray platforms, experimental designs and analysis methods, which made interstudy comparisons almost impossible. Moreover, in most studies experimental designs allowing application of more advanced statistical methods as well as substantial and systematic efforts to validate identified gene expression by a different technique in a large set of independent samples are missing. Based on this survey, a guideline is proposed that may help to increase the validity, reliability and transparency of future studies addressing potential effects of EMF on gene expression.

# **FUTURE DEVELOPMENTS OF TECHNOLOGIES FOR CANCER RISK ASSESSMENT**

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Exposure to electric and magnetic fields has been increasing greatly as countries increase their capacity to generate and distribute electricity and take advantage of the many new technologies, such as telecommunications, to improve lifestyle and work efficiency. Thus, epidemiological investigation on the relation of EMF to the risk of various diseases has been performed over the past two decades. Among all the outcomes evaluated in epidemiological studies of EMF, childhood leukemia in relation to postnatal exposures above 0.3-0.4  $\mu\text{T}$  is the one for which there is most evidence of an association. However there remain a number of uncertainties.

These uncertainties are twofold. The epidemiological studies may have been underpowered since the required large numbers of individuals of precisely defined exposure groups are difficult to obtain. On the other hand the methods applied to detect cancer risk associated changes may lack sufficient sensitivity and comprehensiveness. Omics technologies are expected to fill this gap. However do we have to detect any change of the biological system? It is likely that there is considerable noise. In order to filter this out integrated genomic approaches are currently used to newly define cancers at the molecular level at an unprecedented resolution.

Taking advantage of the Next-Generation Sequencing Technologies an international research consortium plans to comprehensively analyze 25,000 cancer genome samples of 50 different types of cancer. As a result a catalog of all relevant genetic alterations will be available at which future targeted risk assessment studies may focus upon. Similar information will also be generated for epigenetic changes. Furthermore Next-Generation Sequencing facilities may be used to analyse the changes in gene expression induced by several different stresses (e.g. EMF), and to find synergetic genotoxic effects in cells. The improvement of quantitative risk assessment of the impact of EMF exposure, and the elucidation of mechanisms of its complexity is also expected due to these technological advances.

## **FUTURE DEVELOPMENTS OF PROTEOMICS FOR CANCER RISK ASSESSMENT**

*Dr. Ulrich Wacker*

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Proteomics, the parallel analysis of the protein inventory of a cell or tissue is capable to reveal treatment or disease-related alterations in protein content. The composition of the subset of altered proteins together with their direction of expression change can thereby help to elucidate the mechanism as well as the end point of an interaction of a cell or tissue with its environment.

While DNA-analysis reflects what could potentially happen and RNA-analysis reflects what is presently happening in terms of protein expression, proteomics reveals what had actually happened in a cell or tissue on the protein level.

Therefore, in cancer risk assessment as in other fields of biological research various types of proteomics projects are on their way, which more or less follow a basic project design: Proteins from tissue extracts or cells are solubilized and separated on 2D-gels, stained with protein binding dyes and the spot patterns are compared using software assisted image analysis. The subset of variant spots is located within the spot pattern and the size of their alteration is quantified. This variant subset of spots is then excised from the gels, the spots are subjected to in-gel digest and the proteins are identified from their fragments by means of MALDI-TOF or nano-LC-ESI-MS analysis combined with search engine assisted protein database searching.

However, despite its widespread success, this type of basic project design also has certain pitfalls, which are on the one hand caused by the high complexity of the sample i.e. the large number of protein variants of very different protein expression levels, the biological phenomenon of posttranslational modifications as well as by technical limitations.

A plethora of methods has been developed or has been first-time applied as part of proteomic project designs in recent years addressing to improve this basic approach for future applications. A selection of new methods in sample preparation, 2D-gel analysis, mass spectrometric protein identification and succeeding pathway analysis is introduced and assessed with regard to their contribution to either further push the limits of the proteomics approach in general or to improve proteomics based cancer risk assessment.

A project design for establishing a set of protein markers for toxicity is introduced and discussed with regard to its relevance for studying toxin- or radiation induced cancer at early stages.

## **OMICS FOR RISK ASSESSMENT: PATHWAYS TO DISEASE**

*Christopher J. Portier*

*Associate Director, National Institute of Environmental Health Sciences*

There have been a number of papers focused on the use of “omics” data as a tool to “fingerprint” environmental exposures as potentially toxic. Most of these methods rely upon correlation statistics or gene set enrichment analyses to build these hypothetical linkages. In this paper, we will use a method called Structurally-Enhanced Pathway Enrichment Analysis (SEPEA) to build a linkage of cellular signaling/metabolic pathways with human disease based upon human genetic data from the literature. Then, using “omics” data from humans and animals, we build a linkage map between environmental exposures and disease in order to identify the pathways most commonly linked to human disease that appear to have an environmental component to them. Finally, we use these pathways to develop targets for laboratory screening that can be used to guide potential research into the effects of electric and magnetic fields on human health.

## **WHAT HAVE OMICS TAUGHT US ABOUT RISKS ASSOCIATED WITH EXPOSURE TO IONIZING RADIATION?**

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This presentation will review how the various omics technologies have been used to investigate the biological and health impact of ionizing radiation. Exposure to ionizing radiation, as well as other stresses, results in the activation of complex signal transduction pathways, which ultimately influence the response of cells, organs, and organisms. The tools of the postgenomic era enable high-throughput studies of the multiple changes resulting from the interplay of these radiation-signaling pathways. Genomic, transcriptomic and proteomic platforms have revealed important clues into cellular responses at high and low radiation doses as well as different dose rates. Furthermore, the time dependency for these responses is relatively well established and candidate genes identified as potential indicators of individual radiation susceptibility. Polymorphisms in a number of these candidate genes have been investigated to predict normal tissue radiosensitivity and late toxicity, as well as tumor response. Omics technologies are being widely used to predict individual radiation sensitivity and as biomarkers of exposures, both in the clinical setting and in the context of radiological emergencies. Likewise genome-wide approaches are being used to interrogate candidate and whole genome SNP associations to predict therapeutic outcome, late radiation toxicity and the risk of adverse reactions following radiotherapy in cancer patients. The application of metabolomic analysis to induced radiation responses promises to further our understanding of these interrelated stress responses. While there has been significant progress in recent years, the quality and usefulness of some of the data sets are uneven and much remains to be done regarding validation of the data in order to extract meaningful biomarkers. Limitations of the current data and future research directions of omics in radiation risk will be discussed.

## CAN EXTRAPOLATION OF “OMIC” DATA BE APPLIED APPROPRIATELY TO RADIO FREQUENCY FIELD BIOEFFECTS?

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There is a long history, in the ionizing radiation, ultraviolet light, and chemical disciplines, of examining the toxicity, mutagenicity and carcinogenicity of exposure. Radiation biologists performed experiments early on using animals, and attempted to standardize animal models for use in examining toxicity of exposure to whole animals and/or organ systems. When *in vitro* models of cell survival became available, a whole new chapter of ionizing radiation effect studies began. When cell cycle analysis arrived, another wave of ionizing radiation research ensued, as was the case with the subsequent explosion of molecular analysis. Medical oncology attempted to take advantage of *in vitro* models, with an evolving realization that the early efforts to screen thousands of natural and synthetic chemicals for toxicity against a few selected animal and human cancer cell lines were lacking, and that newer models related to human cancer were needed. Screening of initially tens, then hundreds, and potentially thousands of chemicals for mutagenicity, as a flag or guidance for performing more time-consuming and costly animal tumor induction studies for extrapolation to human cancer risk, also evolved. Initially there was the equivalent of an international competition, as different investigators argued through presentations and published papers that their particular mutagenicity model was a better indicator of human chemical carcinogenicity risk than other models being presented and developed. Today, there is a new wave of laboratory investigation and computer analysis, in an effort to link environmental exposures through “omic” studies to human disease. The need for this, when toxic agents are involved, is very obvious, from the standpoints of prevention, treatment to ameliorate the adverse effects after an exposure occurs, and to follow the results and potentially manage treatment. This paper will raise several issues and challenges, existing at least in the near term, to the application of “omics” technologies to investigations of toxicity “resulting” from RF field exposures. These include 1) the level of biological complexity, 2) validation of “omic” systems, 3) transitory responses, 4) necessary but not sufficient responses, 5) relationship (or absence thereof) to a substantiated biological response, and 6) is an “omic” response, by itself, an indicator of an adverse effect or of a beneficial effect?

# USABILITY OF “OMICS”-APPROACH IN RISK ASSESSMENT OF RF-EMF

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We have earlier proposed [1] that transcriptomics and proteomics screening, the so-called “Discovery Science” [2], may be used to reveal molecular targets of RF-EMF and help to understand the possible biochemical mechanism of the RF-EMF-induced effects. Our subsequently published studies have shown that the “omics” approach can be used to reveal changes in gene expression and protein expression and activity (phosphorylation) that seem to be induced in human cells in vitro [3, 4, 5, 6] and in vivo [7] by exposures to RF-EMF.

The first workshop on the applicability of “omics” in RF-EMF research, held in Helsinki in 2005, has produced consensus paper published in 2006 in Proteomics [8]. Conclusions of this consensus paper remain valid. The progress in the area of using transcriptomics and proteomics data to reveal effects of RF-EMF is not limited by the technology but by the number of studies performed and their quality. Database search (EMF-Portal)[9] reveals that in research applicable to RF-EMF, there were published so far only 16 studies: 12 transcriptomics studies, two proteomics studies and two studies where both transcriptome and proteome were analyzed (Table). These studies come predominantly from four research groups in Canada, USA, China and Finland (McNamee, Roti Roti, Xu, Leszczynski). Some of these studies show effects of RF-EMF on “omics” responses whereas the others do not. All of these studies have own limitations. However, such extremely limited “database” does not allow for any far reaching conclusions, except that it is necessary to continue to gather data using transcriptomics and proteomics methods. The presentation will critically review all of the above mentioned 16 published studies.

Published study	omics*
1. Karinen et al. BMC Genomics 2008; 9: 77	P
2. Papparini et al. Bioelectromagnetics 2008: in press	T
3. Chauhan et al. Proteomics 2007; 7: 3896 - 3905	T
4. Zhao et al. Toxicology 2007; 235: 167 - 175	T
5. Zhao et al. Neurosci Lett 2007; 412: 34 - 38	T
6. Whitehead et al. Proteomics 2006; 6: 4739 - 4744	T
7. Zeng et al. Proteomics 2006; 6: 4732 - 4738	T & P
8. Nylund & Leszczynski. Proteomics 2006; 6: 4769 - 4780	T & P
9. Remondini et al. Proteomics 2006; 6: 4745 - 4754	T
10. Qutob et al. Radiat Res 2006; 165: 636 - 644	T
11. Whitehead et al. Radiat Res 2006; 165: 626 - 635	T
12. Belyaev et al. Bioelectromagnetics 2006; 27: 295 - 306	T
13. Lee et al. FEBS Lett 2005; 579: 4829 - 4836	T
14. Nylund & Leszczynski. Proteomics 2004; 4: 1359 - 1365	P
15. Port et al. Int J Rad Biol 2003 ; 79 : 701-708	T
16. Pacini et al. Oncol Res 2002; 13: 19 - 24	T

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2. Aebersold R, Hood LE, Watts JD. Nature Biotechnol. 2000, 18, 359 (commentary)
3. Leszczynski D, Joenväärä S, Reivinen J, Kuokka R. Differentiation 2002, 70:120-129
4. Leszczynski D, Nylund R, Joenväärä S, Reivinen J. Proteomics 2004, 4:426-431.
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9. www.emf-portal.de; standard query - \*omics

## THE RF-EMF CASE II: GENOMICS APPROACH

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The extensive use of mobile phone communication has raised public concerns about adverse health effects of radio frequency (RF) electromagnetic fields (EMFs) in recent years. Although years of research have been spent on this issue by numerous researchers and various hypotheses have been set up and discussed, the underlying mechanism for a non-thermal impact of EMF on a biological system is neither understood on physiological, cellular nor molecular level. Even the existence of such effects is still under discussion.

Classical means of research are usually hypothesis driven: a hypothesis is described, tested and confirmed or disproved. But albeit several explanations on potential biophysical mechanisms are quite comprehensible, none has been verified or is considered generally accepted up to now, and the prediction of effects remains difficult. Epidemiologists face different populations, health issues (lethal and non-lethal) and usage patterns of mobile phones, lab-researchers have to deal with different species, tissues and cell types to be examined: A diversity, making it more than a challenge to correctly predict a reaction of the biological target to the field. Whereas the variety of biological systems and the number of possible effects is manifold, hypothesis driven research can answer only one question at the same time.

In this context, the need for an advanced strategy to formulate new hypotheses becomes evident. A promising approach to search for the mechanical background of field effects would be, to start with systems of reduced complexity, e.g. on molecular and/or cellular level and proceed stepwise by identifying involved proteins, biochemical processes and later tissues or organs that might be affected. Such studies can help to design follow up in vivo studies to verify the previous observations and to investigate their pathophysiological relevance.

Allowing to start without any hypothesis, high-throughput-screening technologies as represented by the various means of 'Omics' methods enable a broadening of views and facilitate the identification of targets. Therefore they are considered as tools that help generating new ideas or hypotheses which might remain obscured when applying conventional methods. Especially genomics techniques, frequently represented by genechip microarray analyses are suitable to start with. They can compare differences in transcription patterns between exposed and non-exposed cells and thus reveal the answer of the biological system to the exposure. This answer is not necessarily related to a pathologically relevant change in cellular physiology, but once uncovered it can guide to involved pathways or functional information to obtain further insight in the quality of the reaction.

The range of application of genechip arrays is typically limited to systems, that are simple enough to allow the detection of individual changes without being corrupted by overlaying effects. But as it is still unclear where or how EMFs could induce changes, investigations on cellular level are no drawback.

Although the application of genomics methods is expensive, time consuming, the evaluation of the large amounts of data is sophisticated and they won't give direct information for risk assessment, they will help finding new endpoints that might not have been predicted by theoretically approaching the question. The talk will overview recent publications on the use of genomics technologies investigating the impact of RF-EMF on biological systems and summarize benefits and problems of their use.

## OMICS AND THE PRECAUTIONARY PRINCIPLE

*Gary Marchant, Ph.D., J.D.*

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Toxicogenomics and related 'omic approaches present a major challenge and opportunity to better define and operationalize the precautionary principle or precautionary approach. At the same time, application of precaution is a critical factor for interpreting and applying toxicogenomic data. Two key questions are at the intersection of omics and precaution:

- (i) what significance (if any) should be given to omic biomarker responses that are not associated with any traditional toxicological endpoint?; and
- (ii) what regulatory or other response is appropriate for any findings that some individuals may be genetically susceptible to a particular exposure, such as radiofrequency from cellular phones?

With respect to the first question, one of the major potential benefits of toxicogenomic and other omic technologies is to provide an earlier, more specific, and more sensitive marker of toxicity. At the same time, it is likely that some exposures may produce or be associated with omic responses that have no toxicological significance because they are simply temporary or adaptive responses to an exposure. The observation of a toxicogenomic response not linked to a known toxicological endpoint therefore presents a dilemma that will be examined through the lens of precaution and the precautionary principle. Similarly, the observation of a small percentage of the population that might be genetically susceptible to a product that is otherwise beneficial for the population at large also creates a dilemma for risk managers and policymakers that again invokes and confronts the precautionary principle. This presentation will examine these dilemmas created by the intersection of omic technologies and the precautionary principle, discuss possible options for resolution, and explicate some of the tensions and trade-offs inherent in both omic technologies and the precautionary principle.