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Toxicogenomics and cancer pathogenesis

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Abstract- Toxicogenomics is emerging field give idea of the application of large-scale differential gene expression data to toxicology, starting to influence drug discovery and development in the pharmaceutical industry. Its future potential in cancer pathogenesis, study of poisons and poisoning and has an ancient and venerable history. Toxicogenomics is the merging of toxicology with technologies that have been developed, together with bioinformatics, to identify and quantify global gene expression changes for gene therapy. Immunotoxic processes and the development of in vitro screening assays is therefore expected to be of value for mechanistic insight into immunotoxicity and hazard identification of existing and novel compounds. Successful application of toxicogenomic approaches, such as DNA microarrays, inextricably relies on appropriate data management, the ability to extract knowledge from massive amounts of data and the availability of functional information for data interpretation. Toxicogenomics is considered a valuable tool for reducing pharmaceutical candidate attrition by facilitating earlier identification, prediction and understanding of toxicities. Pharmaco-epidemiological (toxicogenomics) data is now available for both antiepileptic drugs, evidence for human carcinogenicity. Therapeutic researches converge triad of rejuvenation, regeneration or replacement strategies that rely on self-healing processes, stem cell regeneration organ transplantation. Metastases studies usually display more genetic changes than the primary tumour. Helix-loop-helix application in translational and functional toxicogenomics transcription factors has been implicated in diverse cellular processes such as proliferation, apoptosis, differentiation, and migration.

Introduction

The age of genomics and proteomics has opened up many opportunities in drug research and development. Expectations have been high for the usage of genomics and the benefits that genomics can provide to reinvigorate many stages of drug discovery and development [1]. Toxicogenomics is a new approach to understanding the genetic mechanisms and biochemical pathways to disease by environmental toxicants via the simultaneous analysis of gene and protein expression [2].A useful method for detecting the carcinogenic potential of endocrine active substances (EASs) in the short term with the generation of understanding of mode-of-action and mechanisms when a reliable database with information about proteomics and information technology [3]. Toxicology will be greatly augmented by the application of the knowledge of genetics and researchers have entered a new area of specialty Toxicogenomics i.e., the marriage of toxicology and genomics. Data generated by such research techniques will impact many areas including health and environmental sciences [4]. The evaluation of compounds could best be realized if this promising technology could be implemented in this research is fully anchored in the traditional study end points currently used to characterize phenotypic outcome and to support the safe conduct of clinical testing. Toxicogenomics and related technological biotools to positively impact drug development and guidance has been published [5]. Toxicogenomics is a scientific field, which studies how the genome is involved in responses to environmental stressors, toxicants and in general xenobiotics. It combines studies of genomics, cell and tissue-wide protein expression and metabonomics to understand the role of gene environment interactions in healthy and disorders [6]. Identification of specific gene expression profiles in biological systems associated with xenobiotic exposure and assuming that the expression pattern of a gene product and its function are tightly correlated, this provides insight into the underlying mechanisms of action of toxicants [7]. The application of large-scale differential gene expression (DGE) data to toxicology is starting to influence new drug discovery and development in the pharmaceutical industry. Toxicological pathologists, which play key roles in the development of therapeutic agents, have much to contribute to differential gene expression studies, especially in the experimental design and interpretation phases. The intelligent application of differential gene expression to drug discovery can reveal the

potential for both desired (therapeutic) and undesired (toxic) responses [8].

Toxicogenomics and its application in cancer pathogenesis

Accumulation of genetic changes in a somatic cell is considered essential for the genesis of a cancer and has become clear that not all carcinogens are genotoxic, suggesting that some carcinogens indirectly participate in the generation of genetic changes during carcinogenesis. Research suggests an important role of blocked GJIC in carcinogenesis and that different mechanisms are involved in inhibition of the communication by different agents used. There are multiple nongenotoxic mechanisms in carcinogenesis, and that working hypothesis-oriented approaches are encouraged rather than simple screening of chemicals in developing test systems for the detection of nongenotoxic carcinogens [9]. The identification of toxicological potential in new chemical entities early on in development would be highly desirable in streamlining and reducing the cost of drug development. Studies of tumors profiling and associating these with pathology and phenotype have shown the potential of the technique. Application of the technique in toxicology is only at the preliminary stage [10]. Currently developed gene expression techniques using microarrays in toxicological studies (toxicogenomics) facilitate the interpretation of a toxic compound's mode of action and may also allow the prediction of selected toxic effects based on gene expression changes. The expression profile of the four nongenotoxic carcinogens were compared to the profiles of the four genotoxic carcinogens 2-nitrofluorene (2-NF), dimethylnitrosamine (DMN), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), and aflatoxin B1 (AB1) and showed responses to oxidative DNA or protein damage [11].

Ochratoxin A (OTA) is a mycotoxin occurring naturally in a wide range of food commodities. OTA has raised public health concerns. A reduction in HNF4alpha may be associated with nephrocarcinogenicity. Many Nrf2-regulated genes are involved in chemical detoxication and antioxidant defense. The depletion of these genes is likely to impair the defense potential of the cells, resulting in chronic elevation of oxidative stress in the kidney and could contribute to OTA carcinogenicity [12]. OTA is nephrotoxic produced by fungi and is suspected of being the main etiological agent responsible for human Balkan endemic nephropathy (BEN) and associated urinary tract tumours. OTA is a genotoxic carcinogen by induction of oxidative DNA lesions coupled with direct DNA adducts via quinone formation [13]. Toxicogenomics tools used for studying the genotoxic effect of active compounds on the gene-expression profile of A375 human malignant melanoma cells, through the other molecular functions of target genes, regulatory pathways and mechanisms of malignant melanomas [14]. The safety significance of OTA in food has to rely on animal data, with renal toxicity and carcinogenicity being considered the pivotal effects. Interacting epigenetic mechanisms, including protein synthesis inhibition, oxidative stress and the activation of specific cell signalling pathways, is responsible for OTA carcinogenicity [15]. Particular genotoxic events such as gene mutations or chromosome damage are considered hallmarks of cancer. The genotoxicity testing battery enables relatively by assessing a chemical's ability to cause genetic damage in cells and understanding underlying mechanisms is extremely important for facilitating cancer risk assessment. Recent progress in the development and application of toxicogenomics to the derivation of genomic biomarkers associated with mechanisms of genotoxicity and carcinogenesis [16]. Radiolabeled OTA or MS failed to demonstrate formation of OTA-derived DNA-adducts. Generation of oxidative stress by OTA, the oxidative stress showed the high potency of OTA in rodents. OTA causes disruption of mitosis resulting in blocked or asymmetric cell division. This may present an increased risk of aneuploidy acquisition and may play a critical role in OTA-induced tumor formation [17].

Toxicogenomics History

Toxicology is the study of poisons and poisoning and has an ancient and venerable history. Although there have been numerous notorious poisonings throughout the ages and rather astute descriptions of toxic agents, the scientific study of toxicology did not commence until the 19th century. There was rapid development of analytical methods in the late 19th century and then an acceleration of both method and scientific development in the latter half of the 20th century. Toxicology today can be subdivided into clinical toxicology, forensic toxicology, industrial or occupational toxicology, environmental toxicology, pharmaceutical toxicology, experimental toxicology, and workplace drug testing. Charles Dickens described in his 'Pickwick Papers' subjects with this illness already 150 years ago[18,19]. An integration of clinical and forensic toxicologist society has been working since last 40 years. [20] Emerging technologies in pharmacogenomics and toxicogenomics may identify such markers if well-defined DILI cases and controls can provide tissue samples for analysis [21]. A parallelism exists between human cytogenetics and cytogenetic toxicology. The birth of human cytogenetics occurred in 1956 when it was published that the diploid number of

chromosomes in humans is 46. Human cytogenetics has proven to be effective over its 50-year lifespan because they are cheap, fast, and wide-ranging. In genotoxicology, they continue to be useful to identify mutagenic agents as well as to evaluate and analyze exposed populations [22]. More recent developments have broadened pharmacogenetic approaches to include novel genomic techniques with introduction of the term pharmacogenomics in the 1990's. Genetic and genomic approaches (toxicogenetics and toxicogenomics) are also being applied in the "environmental genome project" [23]. The subsequent emergence of the discipline of molecularize toxicogenomics has required the deliberate development of communication across the myriad disciplines necessary to produce toxicogenomic knowledge; articulation of emergent forms, standards, and practices with extant ones; management of the tensions generated by grounding toxicogenomics [24]. A significant body of proof of principle studies has emerged that demonstrates a range of statistical methodologies applied to predictive toxicology. These studies rely on class prediction methods--mathematical models generated using the gene expression profiles of known toxins from representative toxicological classes--to predict the toxicological effect of a compound based on the similarities between its gene expression profile and the profiles of a given toxicological class [25]. Toxicology is at a crossroads today, at an interface between basic science and applied projects. From its past as a descriptive discipline, it has incorporated a medley of concepts and technology from basic science. The usefulness of many approaches is now being evaluated. The hope is that toxicology will be able to be much more predictive in the future; a great need exists in the pharmaceutical industry [26].

Pharmacology of toxicogenomical gene therapy

Raloxifene (RAL) inhibits the proliferation and induces the apoptosis and G(1) cell cycle arrest via MAPK cascades in human prostate carcinoma cells. Studies about the role of mitogen-activated protein kinase (MAPK) in the apoptosis and cell cycle arrest of human prostate carcinoma cells induced by raloxifene (RAL) demonstrated a dose-dependent proliferation inhibition of RAL in the PC3 cells. A G(1) cell cycle arrest and apoptosis were induced in the PC3 cells exposed to 10(-6) mol/L RAL. 10(-6) mol/L RAL induced the activation of ERK1/2 and p38 with different time courses, but it did not induce the activation of JNK. Up-regulation of P21(WAF1) mRNA expression by activating ERK1/2 and down-regulation of cyclinD1 by activating p38 induces G(1) cell cycle arrest in the human prostate carcinoma cells [27]. Gene therapy vectors based on lentiviruses persist in the host and are ideally suited for long-term therapies of genetic disorders. Recent incidences of T cell leukemia in X-SCID children receiving gene therapy reveal discrepancy among the preclinical and clinical studies. They used cDNA microarray to examine transcriptional changes in BALB/c and CD-1 (IRC) mice, the two common murine strains used in pharmacological and toxicological studies. Gene numbers and gene expression were observed in BALB/c mice, whereby expression of 15 oncogenes was up-regulated in CD-1 (ICR) mice. Distinctive toxicogenomic profiles of two mouse strains should be considered in the context of future development of sensitive models for toxicity evaluation of lentiviral vector products [28]. The recently proposed concept of molecular connectivity maps enables researchers to integrate experimental measurements of genes, proteins, metabolites, and drug compounds under similar biological conditions. The study of these maps provides opportunities for future toxicogenomics and drug discovery applications. The development and application of this computational framework using Alzheimer's Disease (AD) as a primary example in three steps was described. Initial explorations of the AD connectivity map yielded a new hypothesis that diltiazem and quinidine may be investigated as candidate drugs for AD treatment [29]. Negative regulators of basic helix-loop-helix transcription factors, has been implicated in diverse cellular processes such as proliferation, apoptosis, differentiation, and migration. However, the specific role of Id1 in titanium dioxide (TiO2)-induced lung injury has not been investigated. Investigation whether TiO2 induces apoptosis in H1299 lung cancer cells and by which pathways has been done. Based on the results of the LDH assay, dual staining with Annexin V-FITC and propidium iodide (PI), and RT-PCR analysis of apoptosis-related gene expression, the functional role of Id1, cells were transduced with a recombinant adenovirus expressing Id1, and the effects on sensitivity to TiO2 were analyzed [30]. Synthetic polymers and nanomaterials display selective phenotypic effects in cells and in the body signal transduction mechanisms involved in inflammation, differentiation, proliferation, and apoptosis. When physically mixed or covalently conjugated with cytotoxic agents, bacterial DNA or antigens, polymers can drastically alter specific genetically controlled responses to these agents. The pharmacological and toxicological effects of polymer formulations of biological agents, introduces a new field i.e. polymer genomics [31]. Metabolic complications of antiretroviral therapy (ART) have emerged as a major concern for long-term, successful management of HIV infection. Metabolic toxicity of ART suggest that single-nucleotide

polymorphisms (SNPs) in several genes involved in lipid metabolism and lipid transport in the general population (ABCA1, APOA5, APOC3, APOE, CETP) might modulate plasma triglyceride and high-density lipoprotein cholesterol levels in HIV-infected patients, that evaluate the contribution of SNPs in the context of multi-SNP and haplotype analysis, and the validation of genetic markers in independent, large patient cohorts [32]. Toxicogenomics represents the merging of toxicology with technologies that have been developed, together with bioinformatics, to identify and quantify global gene expression changes. It represents a new paradigm in drug development and risk assessment. Toxicogenomics will be increasingly integrated into all phases of the drug development process particularly in mechanistic and predictive toxicology, and biomarker discovery [33]. Assessment of immunotoxicity by gene expression profiling show that microarray analysis is able to detect known and novel effects of a wide range of immunomodulating agents. Besides the elucidation of mechanisms of action, toxicogenomics is also applied to predict consequences of exposing biological systems to toxic agents. It contributes to the understanding of immunotoxic processes and the development of in vitro screening assays, though, and is therefore expected to be of value for mechanistic insight into immunotoxicity and hazard identification of existing and novel compounds [34]. Kojic acid is a natural product and normally used as a food additive and preservative, a skin-whitening agent in cosmetics, a plant growth regulator and a chemical intermediate. Using DNA microarray technology, the overall biological effects of kojic acid on the gene expression profiling of a human skin A375 malignant melanoma cells were examined. Seven down-regulated genes of APOBEC1, ARHGEF16, CD22, FGFR3, GALNT1, UNC5C and ZNF146 that were typically validated by the real-time quantitative PCR (RT-qPCR) analysis technology showed to be the tumor suppressor genes in melanoma cancer cells. The differentially expressed genes may become useful markers of skin malignant melanoma for further diagnostic and therapeutic applications [35]. The non-viral vectors, cationic lipid (CL) formulations are the most widely studied for the delivery of genes, antisense oligonucleotides and gene silencing nucleic acids such as small interfering RNAs. Microarrays analysis to examine the effect of Lipofectin and Oligofectamine on the gene expression profiles of human A431 epithelial cells revealed marked changes in the expression of several genes for both Lipofectin- and Oligofectamine-treated cells. Inadvertent gene expression changes can be induced by the delivery formulation alone and that these may, ultimately, have important safety implications for the use of these non-viral vectors in gene-based therapies [36].

Immunology of toxicogenomics gene therapy

Application of the technique in toxicology is referred to as toxicogenomics. Analysis of immunotoxicity by gene expression profiling show that microarray analysis is able to detect known and novel effects of a wide range of immunomodulating agents and the understanding of immunotoxic processes, the development of in vitro screening assays and hazard identification of existing and novel compounds [37]. Toxicities of polycyclic aromatic hydrocarbons (PAHs) in selected Japanese flounder (*Paralichthys olivaceus*) observed alteration of immune function by the exposure to heavy oil. The microarray detected alteration of immune system-related genes in the kidneys of heavy oil-exposed flounders, including down-regulation of immunoglobulin light chain, CD45, major histocompatibility complex class II antigens and macrophage colony-stimulating factor precursor, and up-regulation of interleukin-8 and lysozyme [38]. The hematopoietic stem and progenitor cell marker, CD34, as a potential marker of hair follicle bulge keratinocytes. Using a CD34-specific antibody, identified intense membrane staining on keratinocytes in the bulge region of the mouse hair follicle. The use of this marker facilitates isolation of live epithelial cells with stem and progenitor cell characteristics, potentially providing a tool for the study of carcinogen target cells, gene therapy and tissue engineering applications [39]. Mercury is a toxic and hazardous metal that occurs naturally in the earth's crust.Consumption of mercury in food, the populations of many areas, particularly in the developing world, have been confronted with catastrophic outbreaks of mercury-induced diseases and mortality and its systemic, immunotoxic, genotoxic/carcinogenic, and teratogenic health effects; and the dietary influences on its toxicity [40]. Exposure to the trichothecene mycotoxin deoxynivalenol (DON) alters immune functions in vitro and in vivo. DON was found to induce the cytokines interleukin (IL)-1alpha, IL-1beta, IL-6 and IL-11.DON upregulated expression of the chemokines macrophage inhibitory protein-2 (MIP-2), cytokine-induced chemoattractant protein-1 (CINC-1), monocyte chemoattractant protein (MCP)-1, MCP-3, and cytokine-responsive gene-2 (CRG-2). c-Fos, Fra-, c-Jun, and JunB, components of the activator protein-1 (AP-1) transcription factor complex, were induced by DON as well as another transcription factor, NR4A1[41, 44].

Genotoxicity strategies

There are two strategies for assessment of the toxicity of complex mixtures for genotoxicity

studies of complex combustion mixtures. The first, a strategy for identifying biologically active compounds or compound classes in complex mixtures, is called bioassay-directed fractionation and characterization. A second strategy, the comparative potency method, provides an approach to evaluating the relative toxicities of a series of mixtures. The comparative mutagenicity and carcinogenicity of a series of combustion emissions has been assessed using dose-response studies in bacteria, mammalian cells, and rodents [45].

With the advent of new technologies (e.g., genomics, automated analyses, and in vivo monitoring), new regulations (e.g., the reduction of animal tests by the European REACH), and new approaches to toxicology (e.g., Toxicity Testing in the 21st Century, National Research Council), the field of regulatory genetic toxicology is undergoing a serious re-examination. Current methods for evaluating mutagenic/genotoxic risk uses standard genotoxicity test batteries, and suggest ways to address and incorporate new technologies [46]. The performance of a battery of three of the most commonly used in vitro genotoxicity tests--Ames+mouse lymphoma assay (MLA)+in vitro micronucleus (MN) or chromosomal aberrations (CA) test--has been evaluated for its ability to discriminate rodent carcinogens and non-carcinogens, from a large database of over 700 chemicals compiled from the CPDB ("Gold"), NTP, IARC. 183 chemicals were identified that were non-carcinogenic after testing in both male and female rats and mice. There were genotoxicity data on 177 of these. The specificity of the Ames test was reasonable (73.9%), but all mammalian cell tests had very low specificity (i.e. below 45%), and this declined to extremely low levels in combinations of two and three test systems. When all three tests were performed, 75-95% of non-carcinogens gave positive (i.e. false positive) results in at least one test in the battery [47]. Current guidelines and recommendations for genotoxicity testing of pharmaceuticals are disparate, both in terms of the most appropriate tests to use and the protocols to follow. A project was undertaken to collect and collate information specifically pertaining to the genotoxicity testing of pharmaceuticals in order to obtain a clear understanding of international strategy and procedures in the pharmaceutical industry [48]. Higher throughput methods, high content analysis and automated screening methods are of highest demand in drug development today. In toxicology, these strategies are becoming increasingly important, as well. The sensitivity, specificity and relevance of the comet assay as a method for determination of DNA damage in vivo and in vitro have been highlighted in many studies. It prove the high reproducibility, flexibility, efficiency and suitability of the procedure as a fully automated analysis method in higher throughput genotoxicity testing in vitro [49]. Genotoxicity of the 2,4-dichlorophenoxyacetic acid (2,4-D) and a commercially-used derivative, 2,4-D dimethylamine salt (2,4-D DMA), was evaluated in CHO cells using SCE and single cell gel electrophoresis (SCGE) assays. The doses of 2,4-D and 2,4-D DMA were equally genotoxic in all of the assays. The results indicate that 2,4-D induces SCE and DNA damage in mammalian cells, and should be considered as potentially hazardous to humans [50]. The effects of ivermectin (IVM) and its commercial formulation ivomec (IVM 1.0%) were studied on Chinese hamster ovary (CHO(K1)) cells by several genotoxicity [sister chromatid exchange (SCE) and single cell gel electrophoresis (SCGE)] and cytotoxicity [cell-cycle progression (CCP), mitotic index (MI), proliferative replication index (PRI), 3(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and neutral red (NR)] bioassays within the 1.0-250 microg/ml concentration-range. NR and MTT assays revealed a cell growth inhibition when 0.25-250.0 microg/ml of both compounds was employed. The results highlighted that IVM and ivomec exert both genotoxicity and cytotoxicity in mammalian cells in vitro, at least in CHO(K1) cells [51]. In vitro genotoxicity assays are often used to screen and predict whether chemicals might represent mutagenic and carcinogenic risks for humans. Recently discussions have focused on the high rate of positive results in in vitro tests, especially in those assays performed in mammalian cells that are not confirmed in vivo. Recommendations to improve testing included: (1) re-examine the maximum level of cytotoxicity currently required for in vitro tests; (2) re-examine the upper limit concentration for in vitro mammalian studies; (3) develop improved testing strategies using current in vitro assays; (4) define criteria to guide selection of the appropriate follow-up in vivo studies; (5) develop new and more predictive in vitro and in vivo tests. The current paradigm in toxicogenomics moves from a hazard identification approach to a "realistic" risk-based approach that incorporates information on mechanism of action, kinetics, and human exposure [52, 53]. Non-relevant metabolites are degradation products of AIs, which do not or only partially retain the targeted toxicities of AIs. For non-relevant metabolites without genotoxicity, the application of the concept of "thresholds of toxicological concern" results in a health based drinking water limit of 4.5 mug/L even for Cramer class III compounds, using the TTC threshold of 90 mug/person/day and the risk assessment process applies large minimal margins of exposure (MOEs) to compensate for the shorter duration of the studies. The results of the targeted toxicity testing will provide a science basis for setting tolerable drinking water limits for

"non-relevant metabolites" based on their toxicology [54].

Toxicogenomics and DNA microarrays

New era bioinformatics capability is widely acknowledged as central to realizing the promises of toxicogenomics. Successful application of toxicogenomic approaches, such as DNA microarrays, inextricably relies on appropriate data management and the availability of functional information for data interpretation. Microarray data management and analysis software, called Array-Track, that is also used in the routine review of genomic data submitted to the FDA. ArrayTrack is publicly online (http://www.fda.gov/nctr/science/centers/toxicoinformatics/ArrayTrack/index.htm) [55]. Aquatic organisms, Daphnia magna has been used extensively to evaluate organism- and population-level responses of invertebrates to pollutants in acute toxicity or reproductive toxicity tests. The DNA microarray was used to evaluate gene expression profiles of neonatal daphnids exposed to several different chemicals: Copper sulfate, hydrogen peroxide, pentachlorophenol, or beta-naphthoflavone [56]. DNA microarrays are rapidly becoming one of the tools of choice for large-scale toxicogenomic studies. Compounde in ecotoxicological studies and its expression profiling may subsequently find utility in ecotoxicological-based computer simulation models, such as the Biotic Ligand Model (BLM), in which gene expression information may be integrated with geochemical, pharmacokinetic, and physiological data to accurately assess and predict toxicity of metals to aquatic organisms [57]. *Escherichia coli* gene-deletion mutants specific to DNA repair and damage signaling pathways, and each bar-coded mutant can be tracked in pooled format using bar-code specific microarrays. Microarray-based screens were used for en masse identification of individual mutants sensitive to methyl methanesulfonate (MMS). DeltadinG cells filament in response to MMS they exhibit wild-type sulA expression after exposure and SulA levels suggests that DinG is associated with the SulA-independent filamentation pathway [58]. MARS (Microarray Analysis and Retrieval System) provides a comprehensive MIAME supportive suite for storing, retrieving, and analyzing multi color microarray data. MARS is fully integrated into an analytical pipeline of microarray image analysis, normalization, gene expression clustering, and mapping of gene expression data onto biological pathways. Microarray based research projects using a unique fusion of Web based and standalone applications connected to the latest J2EE application server technology. More information can be found at (http://genome.tugraz.at.) [59]. Gene arrays are valuable tools in the identification of differentially expressed genes and potentially identify new gene biomarkers altered by exposure of organisms to xenobiotic compounds, either singly or as complex mixtures.The mechanisms of interaction between estrogen receptor (ER) and aryl hydrocarbon receptor (Ah receptor or AhR) signalling pathways using toxicogenomic approaches. Inhibition of AhR with ANF did not reverse the effect of TCB on ER-mediated transcription suggesting that AhRs do not have a direct role on TCB-mediated decreases of ER-mediated responses and found to be affected in our targeted SalArray chip that are important for the reproductive effects of endocrine disruptors [60]. Toxicogenomics study of the MicroArray Quality Control (MAQC) project empirically revealed that the DEGs selected using a fold change (FC)-based criterion were more reproducible than those derived solely by statistical significance such as P-value from a simple t-tests and FC-based ranking coupled with a nonstringent P-value cutoff is used for gene selection compared with selection based on P-value based ranking method. Identified that the MAQC recommendation should be considered when reproducibility is an important study objective [61].

Toxicogenomics and clinical mechanisms

Toxicogenomics is considered a valuable tool for reducing pharmaceutical candidate attrition by facilitating earlier identification, prediction and understanding of toxicities. A retrospective evaluation of 3 years of routine transcriptional profiling in non-clinical safety studies was undertaken to assess the utility of toxicogenomics in drug safety assessment, 33 compounds were shown to be a robust biomarker for dosages considered to be toxic and 40% of targets profiled [62]. R7199 caused hepatic steatosis in rats, no hepatotoxicity was observed with R0074. The induction of Cyp 2B and Cyp 3A1 directly correlates to the findings in the livers of treated animals. The effects on genes of the steroideogenic pathway relate to the deregulation of cholesterol homeostasis. Observed the inhibition of beta-oxidation, indicating impaired lipid metabolism [63-65]. Exposures of dibutyl phthalate were examined in male and female F344/N rats and B6C3F1 mice in 13-week feed studies. Hepatomegaly (increased relative liver weight) was observed in males in all exposed groups and in females receiving 2,500 ppm or greater. No gross lesions were observed at necropsy. Moderate hypospermia of the epididymis was diagnosed in all male rats in the 7,500 and 10,000 ppm groups; mild hypospermia of the epididymis was diagnosed in 2 of 10 males in the 5,000 ppm group [66]. The use of gene expression profiling in both clinical and laboratory settings would be enhanced by better characterization of variance due to individual,

environmental, and technical factors. The study factors that emerged as key sources of variability included gender, organ section, strain, and fasting state [67]. Butterbur extracts (*Petasites hybridus*) are recommended for the prevention of migraine, but pharmacovigilance reports may be suggestive for rare hepatobiliary toxicity.In a 28-day toxicity study at approximately 200-fold of therapeutic doses induced liver transaminases and bilirubin elevations were observed. In a subsequent 6-months chronic toxicity study the initial hepatobiliary effects were reproduced, but at the end of the study liver function recovered. Liver function in vitro at >170-fold of therapeutic C(max) levels, including cytotoxicity (LDH, MTT, ATP), transaminase activities (ALT, AST), albumin synthesis, urea and testosterone metabolism to assay for CYP monooxygenase activity [68]. Toxicogenomics has emerged as use of genome-scale mRNA expression profiling to monitor responses to adverse xenobiotic exposure. Toxicogenomics is being investigated for use in the triage of compounds through predicting potential toxicity, defining mechanisms of toxicity, and identifying potential biomarkers of toxicity. The analysis of gene expression data from preclinical studies to find differentially expressed genes that correlate with pathology (coincident biomarker) or precede pathology (leading biomarker) within a lead series; or gene expression profiling can be performed directly on the blood from preclinical studies or clinical trials to find biomarkers that can be obtained noninvasively [69].

Drug resistance mechanisms and carcinogenicity

There has been considerable debate about the relationship between epilepsy and cancer, in particular whether the incidence of cancer is increased in people with epilepsy and whether antiepileptic drugs promote or protect against cancer. Available evidence from animal experiments, genotoxicity studies and clinico-epidemiological observations, and discuss proposed mechanisms underlying the association between epilepsy and cancer. Early human epidemiological studies found an association between phenobarbital and hepatocellular carcinoma, and several subsequent studies suggested an association with lung cancer. Phenytoin has been causally implicated in three human cancers: lymphoma, myeloma and neuroblastoma, the latter specifically in the setting of foetal hydantoin syndrome. Despite considerable long-term pharmaco-epidemiological data being available for both antiepileptic drugs, evidence for human carcinogenicity is not consistent and both are considered only possibly carcinogenic to humans [70]. Olivacine derivative S16020-2 (NSC-659687) is a DNA topoisomerase II inhibitor endowed with a remarkable antitumor activity against various experimental tumors. From the Chinese hamster lung fibroblast cell line DC-3F, a subline resistant to S16020-2, named DC-3F/S16, was selected by adding stepwise increasing concentrations of the drug to the cell growth medium. Topoisomerase IIalpha was expressed at the same level in both sensitive and resistant cells, whereas expression of the beta-enzyme was approximately 50% lower in the resistant cells. This amino acid substitution in a highly conserved sequence of the enzyme appears to be responsible for the resistance to S16020-2 [71]. Cross-resistance is an important issue for the evaluation of new antiestrogens to treat advanced breast cancer patients who have failed tamoxifen therapy. In addition, postmenopausal patients treated with long-term adjuvant tamoxifen show a 3-4-fold increase in the risk of developing endometrial cancer. The effects of the new tamoxifen analogue GW5638 on breast and endometrial cancer growth were studied. GW5638 did not promote tumor growth, and was effective in blocking the effects of postmenopausal estradiol on the growth of tamoxifen-naïve breast and endometrial tumors. GW5638 could be developed as a second line agent for advanced breast cancer patients and an important first line agent to evaluate as an adjuvant treatment or chemopreventive [72]. Arsenic toxicity is dependent on its chemical species. In humans, the bladder is one of the primary target organs for arsenic-induced carcinogenicity. Study aimed at comparing the toxic effect of DMMTA(V) with that of inorganic arsenite (iAs(III)) on cell viability, uptake efficiency and production of reactive oxygen species (ROS) toward human bladder cancer EJ-1 cells. iAs(III) was known to be toxic to most cells [73].The physicochemical model of iodine-lithium-alpha-dextrin (ILalphaD) is based on the human blood and the stereochemistry of moving equilibred systems of dynamically balanced organic polymers conformation complexed with the iodine and lithium molecules. ILalphaD therapy contributes to anti-HIV and anti-inflammatory effects, resolution of dermatological and neurological pathology and dramatically improves the quality of life reflecting on enhanced treatment adherence [74]. Laboratory models for breast and endometrial cancer have had an enormous impact on the clinical development of antiestrogens. The DMBA-induced rat mammary cancer model has provided the scientific principles required to evaluate long-term adjuvant tamoxifen therapy. The success of tamoxifen as an agent that preserves bone density, lowers cholesterol and prevents contralateral breast cancer has become a classic example of a multimechanistic drug. The laboratory studies of raloxifene provided the scientific rationale for the use of raloxifene as a preventive for osteoporosis

[75]. Tamoxifen has mixed agonist/antagonist activities, leading to tissue-specific estrogen-like actions and endometrial cancer. The purpose of this study was to evaluate the effects of antiestrogens on the growth of estrogen receptor (ER)-positive ECC-1 endometrial cancer cells in vitro and in vivo. Growth studies and luciferase assays using ERE-tK and AP-1 reporters was done. ERalpha, ERbeta, EGFR, and HER2/neu mRNAs were determined by RT-PCR. E2 and raloxifene down regulated ERalpha protein; in contrast, 4OHT did not. ICI182,780 completely degraded the receptor. Tamoxifen and raloxifene are antiproliferative agents and antiestrogens in ECC-1 endometrial cells in vitro and in vivo [76].

Aflatoxin B1 (AFB1) a secondary metabolite of Aspergillus flavus and A. parasiticus, is known for its carcinogenicity and immunosuppressive effects. The study concerning the involvement of the hypothalamus-pituitary-adrenal gland axis in the immunosuppressive effects of AFB1 in C57Bl/6 mice were done. The dose of AFB1 for the immunosuppressive effects on blastogenic response, IL-2 production, and primary antibody production of splenic cells was much higher than previous studies involving other mice strains. AFB1 decreased the amount of circulating anti-SRBC antibody, and the helper-T cell and B cell populations in phenotyping splenic lymphocytes. There were no significant changes in natural killer cell activity, mixed lymphocyte response, hypothalamic biogenic amine concentrations, and corticotropin releasing factor, and of adrenocorticotropic hormone and corticosterone in plasma. The hypothalamic-pituitary-adrenal axis does not appear to have a major role in AFB1-induced immunotoxicity [77].

Drug toxicogenomics

Recently toxicogenomics approaches were use to better understand the hepatotoxic potential of human pharmaceutical compounds and to assess their toxicity earlier in the drug development process by means of a toxicity screen [78]. Drug-induced liver injury (DILI) due to a particular medication or herbal and dietary supplement relies on clinical history, exclusion of competing causes, prior reports of DILI, and judgment-no objective laboratory or histological tests exist to confirm a diagnosis of suspected DILI. Developing technologies in pharmacogenomics and toxicogenomics may identify such markers if well-defined DILI cases and controls can provide tissue samples for analysis [79]. Macrophage activators (MA), peroxisome proliferators (PP), and oxidative stressors/reactive metabolites (OS/RM) all produce oxidative stress and hepatotoxicity in rats. The differential gene responses largely reflect differential activation of transcription factors: MA activate Stat-3 and NFkB, PP activate PPARa, and OS/RM activate Nrf2. Hepatotoxicants were categorize over 100 paradigm compounds as to their oxidative stress potential in rat liver [80]. Effect of the ecotoxicity of silver nanoparticles (AgNPs) in *Caenorhabditis elegans* using survival, growth, and reproduction, as the ecotoxicological endpoints, as well as stress response gene expression studied. Functional genomic studies using mutant analyses suggested that the sod-3 and daf-12 gene expressions may have been related to the AgNPs-induced reproductive failure in C. elegans and that oxidative stress may have been an important mechanism in AgNPs toxicity [81]. Toxicogenomics is becoming a generally accepted approach for identifying chemicals with potential safety problems. Analyzed 33 nephrotoxicants and 8 non-nephrotoxic hepatotoxicants to elucidate time- and dose-dependent global gene expression changes associated with proximal tubular toxicity. Gene expression profiles were generated from kidney RNA by using Affymetrix GeneChips and analyzed in conjunction with the histopathological changes. The gene list contains well-known biomarkers, such as Kidney injury molecule 1, Ceruloplasmin, Clusterin, Tissue inhibitor of metallopeptidase 1, and also novel biomarker candidates [82]. The specific role inhibitor of differentiation (Id1) family of genes in titanium dioxide (TiO2)-induced lung injury has not been investigated. The LDH assay, dual staining with Annexin V-FITC and propidium iodide (PI), and RT-PCR analysis of apoptosis-related gene expression, TiO2 caused a dose- and time-dependent decrease in cell viability and appeared to involve both necrosis and apoptosis. Indicate that Id1 expression attenuates the degree of TiO2-induced cytotoxicity in lung cells [83]. The triazole antifungals myclobutanil, propiconazole and triadimefon cause varying degrees of hepatic toxicity and disrupt steroid hormone homeostasis in rodent in vivo models. Differentially expressed genes included the Phase I xenobiotic, fatty acid, sterol and steroid metabolism genes Cyp2b2 and CYP2B6, Cyp3a1 and CYP3A4, and Cyp4a22 and CYP4A11; Phase II conjugation enzyme genes Ugt1a1 and UGT1A1; and Phase III ABC transporter genes Abcb1 and ABCB1. Gene expression changes caused by all three triazoles in liver and hepatocytes were concerned [84].

Therapeutic and Experimental Strategy

Therapeutic repair encompasses the converging triad of rejuvenation, regeneration or replacement strategies that rely on self-healing processes, stem cell regeneration, and organ transplantation. Natural healing or rejuvenation exemplify inherent, baseline repair secured by tissue self-renewal and de novo cell biogenesis, particularly effective in organs with a high endogenous reparative capacity. Translation into clinical applications requires the establishment of

a regenerative medicine community of practice capable to bridge discovery with personalized treatment solutions [85]. Arteriogenesis, endogenous process is a natural compensation mechanism against stenosis or arterial occlusion-induced tissue hypoperfusion via improvement of blood distribution in the pre-existent collateral arteries. The main chronic artery disorders like coronary heart disease, peripheral artery disease and cerebrovascular disease were extensively studied for angiogenesis and arteriogenesis during the last decade. The adaptive arteriogenesis in the heart, brain and periphery can be stimulated by different chemokines and growth factors. The therapeutic application of these substances resulted in promising data in pre-clinical animal models, i.e. improved collateral conductance, extended neo-vascularization in the collateral dependent tissue regions, decreased infarct area after hemodynamic stroke and better functional parameters in myocardial ischemia [86]. A strategy for screening new monoclonal antibodies (mAb) that could be appropriate for clinical application in oncology, evaluated the suitability of three methods: a direct internalization assay (DIA), an indirect internalization assay (IIA) and an indirect cytotoxicity assay (ICA), by applying them to already selected mAb. The latter were directed against three antigenic systems (38-kDa glycoprotein (gp38), epidermal growth factor receptor, and the neu oncogene product), which, according to their tumor selectivity, could be considered suitable for mAb-guided therapy [87, 88]. A therapeutic strategy was designed to eliminate the humoral immune response to acetylcholine receptor (AChR) in ongoing experimental autoimmune myasthenia gravis (EAMG). Rats with EAMG were treated consisting of three components: (1) A single high dose of cyclophosphamide (200 mg/kg) was used to produce a rapid and sustained fall in the anti-AChR antibody levels by preferential destruction of antibody-producing B-lymphocytes. "Memory" lymphocytes were not eliminated by cyclophosphamide. (2) Irradiation (600 rads) was used to eliminate the "memory" cells. It eliminated the anamnestic response to a challenge with the antigen AChR. (3) Bone marrow transplantation was used to repopulate the hematopoietic system after the otherwise lethal dose of cyclophosphamide. Rats with EAMG treated with this combined protocol showed a prompt and sustained fall in the anti-AChR antibody levels and had no anamnestic response to a challenge with AChR [89]. Alcohol abuse occurs in association with anxiety, depression, or schizophrenia, treatment with the anxiolytic, antidepressant, and neuroleptic drugs, respectively, may facilitate the alcoholic's ability to participate in other programs. Patients should receive drugs that are appropriate to treatment goals as well as to their psychosocial status. Even if a drug therapy is shown to be efficacious under controlled experimental conditions, its effectiveness may be compromised by a large number of factors that include poor compliance by the patient, a lack of a treatment strategy, or failure to optimize the treatment conditions [90]. Lymphoid cells were thought to be uniquely susceptible to excess 2'-deoxyadenosine (dAdo), when exposed to inhibitors of adenosine deaminase (ADA). Human monocytes are as sensitive as lymphocytes to dAdo or to the ADA-resistant congener 2-chloro-2'-deoxyadenosine (CldAdo). Human monocyte function and survival to CldAdo in vitro, together with the monocyte depletion in patients receiving CldAdo chemotherapy, suggests that CldAdo or other dAdo analogues offer a novel therapeutic strategy for chronic inflammatory and autoimmune diseases characterized by inappropriate monocyte deployment or function [91]. Slide-based cytometry (SBC) and related techniques offer unique tools to perform complex immunophenotyping, thereby enabling diagnostic procedures at very early disease stages. Multicolor or polychromatic analysis of cells by SBC is of special importance, not only as a cytomics technology platform but also for patients with low blood volume such as neonates. Predictive medicine aims at the detection of changes in patient's state before the manifestation of the disease or its complications. Such instances concern multiorgan failure in sepsis or noninfectious posttraumatic shock in intensive care patients or the pretherapeutic identification of high-risk patients undergoing cancer cytostatic therapy. Regenerative medicine and tissue engineering apply the principles of cell transplantation, material science, and bioengineering to construct biological substitutes that will restore and maintain normal function in diseased and injured tissues. Neovascularization is promoted by bone marrow-derived endothelial progenitor cells that lead to the formation of entirely new vessels into ischemic tissue [92]. The pandemic of chronic degenerative diseases associated with aging demographics mandates development of effective approaches for tissue repair. As diverse stem cells directly contribute to innate healing, the capacity for de novo tissue reconstruction harbors a promising role for regenerative medicine. Through strategies of replacement to implant functional tissues, regeneration to transplant progenitor cells or rejuvenation to activate endogenous self-repair mechanisms, the overarching goal of regenerative medicine is to translate stem cell platforms into practice and achieve cures for diseases limited to palliative interventions [93].

Tumours and metastases

The prevailing models of cancer metastasis postulate that, after a series of accumulating genetic and epigenetic changes during transformation and invasive growth, the most advanced clone within a primary tumour acquires the critical cellular phenotype enabling dissemination and metastasis. This postulate is particularly based on observations that metastases usually display more genetic changes than the primary tumour [94]. Hepatic surgery is presumed to improve survival of patients with liver metastases (LM) from neuroendocrine tumours (NET). LM-specific variables that could be used as additional selection criteria for aggressive treatment. Three growth types of LM were identified radiologically: single metastasis (type I), isolated metastatic bulk accompanied by smaller deposits (type II) and disseminated metastatic spread (type III). The three groups differed significantly in terms of chronological presentation of LM, hormonal symptoms, Ki-67 index, 5-hydroxyindoleacetic acid and chromogranin A levels, lymph node involvement, presence of bone metastases and treatment options [95]. A transplantable malignant tumor of the rabbit an index of malignancy has been developed which has proven useful in evaluating the severity of the disease for the purpose of statistical investigations in primary tumor and the distribution of metastases in this experimental tumor. From this analysis it is concluded (1) that the severity of the disease as a whole is very irregular among individuals of a series and from series to series, and (2) that the relationship between the extent of the primary lesions and that of the secondary lesions is in constant among individuals [96]. Argentaffine tumor of the appendix has been presented case of a multicentric argentaffine tumor of the cecum with metastases to the liver. This tumor is a carcinoma arising from the Kultschitsky cell of the intestinal epithelium. All argentaffine tumor are slow growing but malignant tumors. A good prognosis is presented after surgical intervention [97]. Blood-borne tumour metastases may be influenced by many factors and determining factors is the number of embolic tumor cells circulating in the blood stream. For these purposes, different doses of viable Ehrlicli ascites tumor cells were injected into the tail vein of mice of both sexes. The incidence and number of lung metastases in each group was determined by actual count and the weights of the lungs, spleen, liver and kidneys were used to establish a quantitative index of the response of the reticulo-endothelial system to the presence of tumor metastases [98]. The Gastrointestinal stromal tumors (GISTs) are rare neoplasms of the gastrointestinal tract. One to three percent of GISTs occur in the esophagus. GISTs have a great potential for diffuse intra-abdominal spread and liver metastasis, which are the two most common modes of dissemination. Metastases to other sites, especially the bones and lung, are relatively rare. Never has an esophageal GIST been documented to present with pulmonary and bone metastases. Metastasis should be considered in any case of an esophageal GIST with suspicious pulmonary or bone lesions [99]. The external pH of solid tumors is acidic as a consequence of increased metabolism of glucose and poor perfusion. Acid pH has been shown to stimulate tumor cell invasion and metastasis in vitro and in cells before tail vein injection in vivo. Oral NaHCO3 selectively increased the pH of tumors and reduced the formation of spontaneous metastases in mouse models of metastatic breast cancer. This treatment regimen was shown to significantly increase the extracellular pH, but not the intracellular pH of tumors by 31P magnetic resonance spectroscopy. NaHCO3 therapy also reduced the rate of lymph node involvement, yet did not affect the levels of circulating tumor cells and shown that oral bicarbonate therapy significantly reduced the incidence of metastases in experimental models of breast and prostate cancer and that the effect seems to be primarily on distal (i.e., colonization), rather than proximal (i.e.,intravasation), processes [100].

Translational and functional toxicogenomics

The inhibitor of differentiation (Id) family of genes, which encodes negative regulators of basic helix-loop-helix transcription factors, has been implicated in diverse cellular processes such as proliferation, apoptosis, differentiation, and migration. The specific role of Id1 in titanium dioxide (TiO2)-induced lung injury has not been investigated.The results of the LDH assay, dual staining with Annexin V-FITC and propidium iodide (PI), and RT-PCR analysis of apoptosis-related gene expression, TiO2 caused a dose- and time-dependent decrease in cell viability and appeared to involve both necrosis and apoptosis. Results indicate that Id1 expression attenuates the degree of TiO2-induced cytotoxicity in lung cells [101]. RASSF1A is a recently identified 3p21.3 tumor suppressor gene. The high frequency of epigenetic inactivation of this gene in a wide range of human sporadic cancers including non-small cell lung cancer (NSCLC) and neuroblastoma suggests that RASSF1A inactivation is important for tumor development. Protein analysis of six genes i.e., ETS2, Cyclin D3, CDH2, DAPK1, TXN, and CTSL, showed that the changes induced by RASSF1A at the RNA level correlated with changes in protein expression in both non-small cell lung cancer and neuroblastoma cell lines [102]. Rapamycin (mTOR) pathway is essential for both growth and differentiation of mammary epithelial cells and

that the action of mTOR is mediated through the induction of the helix-loop-helix transcriptional regulators Id1 and Id2. Rapamycin treatment of HC11 cells resulted in a suppression of Id1 expression and an inhibition of proliferation. Rapamycin also prevented the induction of Id2 by lactogenic hormones and milk protein gene expression [103]. Basic helix-loop-helix E proteins are transcription factors that play crucial roles in T cell development by controlling thymocyte proliferation, differentiation and survival. E protein functions can be repressed by their naturally occurring inhibitors, Id proteins (Id1-4). Transgenic expression of Id1 blocks T cell development and causes massive apoptosis of developing thymocytes, the target genes regulated by E proteins and Id1 expression diminished RORgammat mRNA levels in T cell lines and primary thymocytes while induction of E protein activity restored RORgammat expression [104]. Survivin is expressed in most tumor cells and has been associated with both anti-apoptosis and mitotic progression. The expression and regulation of survivin in the nitric oxide (NO)-exposed human lung carcinoma cells were investigated. The lung carcinoma cell lines CL3, H1299, and A549 but not normal lung fibroblast expressed high levels of survivin proteins. The cdc25 phosphatase inhibitors (Cpd 5 and NSC 663284) and the cdc2 kinase inhibitors (alsterpaullone and purvalanol A) enhanced SNP-induced cytotoxicity and the decrease in survivin expression. Overexpression of survivin by a pOTB7-survivin vector reduced SNP-induced cell growth inhibition and cytotoxicity. The specific p38 MAP kinase inhibitor, SB202190, significantly decreased the cytotoxicity. Anticancer agents including quercetin, arsenite, and cisplatin but not genistein increased the levels of survivin protein [105]. Overexpression of Bcl-2 family members as well as deregulated apoptosis pathways are known hallmarks of lung cancer. Non-small cell lung cancer (NSCLC) cells are typically resistant to cytotoxic chemotherapy and approaches that alter the balance between pro-survival and pro-death Bcl-2 family members have shown promise in preclinical models of NSCLC. Evaluated the effect of GX15-070 and correlated the effect on EGFR status as well as Bcl-2 family protein expression. Show that GX15-070 can disrupt Mcl-1:Bak interactions in lung cancer cells. Identified differential sensitivity of a panel of lung cancer cells to GX15-070 and no clear relationship existed between EGFR status or Bcl-2 family protein expression and sensitivity to GX15-070. Observed synergy between GX15-070 and cisplatin in NSCLC cells. Based on these results, GX15-070 can trigger apoptosis in NSCLC cells and can enhance chemotherapy-induced death [106-108].

References

[1] PeterG.Lord,Alex Nie and Michael Mc Millian (2005) Basic *& Clinical Pharmacology & Toxicology*,98,537–546.

[2] James K. Selkirk and Tennant R.W. (2003) *Pure Appl.Chem. Nos.*11–12, pp.2413–2414.

[3] Tomoyuki Shirai and Makoto Asamoto (2003) *Pure Appl.Chem Nos.*11–12, pp.2419–2422.

[4] Kenneth Olden, Neysa Call, Bruno Sobral and Robin Oakes (2004) *Environmental Health Perspectives*,112:805–807.

[5] Frank D.,Sistare and Joseph J.DeGeorge Methods in Molecular Biology,vol.460.

[6] Laura Vass, János Z. Kelemen, Liliána Z. Fehér, Zsolt Lorincz, Sándor Kulisándor Cseh, György Dormán And László G., Puskás (2008) *International Journal Of Molecular Medicine* 23: 65-74.

[7] Kirsten A. Baken , Rob J., Vandebriel, Jeroen L.A. Pennings,Jos C. Kleinjans,Henk van Loveren (2006) *Elsevier Inc* , doi:10.1016 / j.ymeth. 2006.07.010.

[8] Gary A. Boorman,Steven P. Anderson,Warren M. Casey,Roger H. Brown,Lynn M. Crosby (2002) *Toxicologic Pathology*, 30(1), 15-27.

[9] Yamasaki H., Ashby J., Bignami M., Jongen W., Linnainmaa K, Newbold R.F., Nguyen-Ba G, Parodi S., Rivedal E., Schiffmann D., Simons J.W., Vasseur P. (1996) *International Agency for Research on Cancer, Lyon, France ,Mutat Res.*;3531-2:47-63.

[10] Gant T.W. (2003) *Drug News Perspect*;164:217-21.

[11] Ellinger-Ziegelbauer H., Stuart B., Wahle B., Bomann W., Ahr H.J.(2005) *Mutat Res.*;5751-2:61-84.

[12] Marin-Kuan M., Nestler S., Verguet C., Bezençon C., Piguet D., Mansourian R., Holzwarth J., Grigorov M., Delatour T., Mantle P., Cavin C., Schilter B. (2006) *Toxicol Sci*;891:120-34.

[13] Pfohl-Leszkowicz A., Manderville R.A. (2007) *Mol Nutr Food Res*;511:61-99.

[14] Cheng S.L., Huang-Liu R., Sheu J.N., Chen S.T., Sinchaikul S., Ts G.J. (2007) *Pharmacogenomics*:1017-36.

[15] Marin-Kuan M., Cavin C., Delatour T., S.chilter B. (2008) *Toxicon;*522:195-202.

[16] Ellinger-Ziegelbauer H., Aubrecht J., Kleinjans J.C., Ahr H.J. (2009) *Toxicol Lett*;1861:36-44.

[17] Mally A, Dekant W. (2009) *Mol Nutr Food Res*;534:467-78.

[18] Mathis J.(1995) *Praxis Bern* 1994;8450:1479-85.
[19] Pappas A.A., Massoll N.A., Cannon D.J. (1999) *Ann Clin Lab Sci.*;294:253-62 .
[20] Kłys M. (2005) *Przegl Lek*;626:326-33.
[21] Hayashi P.H. (2009) *Semin Liver Dis*:348-56.
[22] Garcia-Sagredo J.M. (2008) *Biochim Biophys Acta.*;17796-7:363-75.
[23] Motulsky A.G.(2002) *Med Secoli.*;143:683-705.
[24] Shostak S. (2005) *Soc Stud Sci.;*353:367-403.
[25] Maggioli J., Hoover A., Weng L. (2006)*J Pharmacol Toxicol Methods.*;531:31-7.
[26] Guengerich F.P. (2004) *Drug Metab Rev.*;363-4:475-86.
[27] Zhang Y.X., Kong C.Z. (2008) *Zhonghua Yi Xue Za Zhi.*;884:271-5.
[28] Zhao Y., Keating K., Thorpe R. (2007) *Toxicol Appl Pharmacol.* ;2252:189-97.
[29] Li J., Zhu X., Chen J.Y. (2009*) PLoS Comput Biol.*;57:e1000450.
[30] Lee Y.S., Yoon S., Yoon H.J., Lee K., Yoon H.K., Lee J.H., Song C.W. (2009) *Toxicol Lett.*;1893:191-9.
[31] Kabanov A.V. (2006) *Adv Drug Deliv Rev.*;5815:1597-621.
[32] Tarr P.E., Telenti A. (2007) *Antivir Ther.*;127:999-1013.
[33] Guerreiro N., Staedtler F., Grenet O., Kehren J., Chibout S.D. (2003) *Toxicol Pathol.*;315:471-9.
[34] Baken K.A., Vandebriel R.J., Pennings J.L., Kleinjans J.C., van Loveren H. (2007) Methods.;411:132-41.
[35] Cheng S.L., Huang Liu R., Sheu J.N., Chen S.T., Sinchaikul S., Tsay G.J. (2006) *Biol Pharm Bull.*;294:655-69.
[36] Omidi Y., Hollins A.J., Benboubetra M., Drayton R., Benter I.F., Akhtar S. (2003) *J Drug Target.*;116:311-23.
[37] Baken K.A., Vandebriel R.J., Pennings J.L., Kleinjans J.C., van Loveren H.(2007) *Methods*;411:132-41.
[38] Nakayama K., Kitamura S., Murakami Y., Song J.Y., Jung S.J., Oh M.J., Iwata H., Tanabe S. (2008) *Mar Pollut Bull.*;576-12:445-52.
[39] Trempus C.S., Morris R.J., Bortner C.D., Cotsarelis G., Faircloth R.S., Reece J.M., Tennant R.W. (2003) *J Invest Dermatol*;1204:501-11.
[40] Tchounwou P.B., Ayensu W.K., Ninashvili N., Sutton D. (2003) *Environ Toxicol*;183:149-75.
[41] Kinser S., Jia Q., Li M., Laughter A., Cornwell P., Corton J.C., Pestka J. (2004) *J Toxicol Environ Health A.*;6718:1423-41.
[42] Baken K.A., van Loveren H., Pennings J.L., de Vries A., Breit T.M., van Steeg H. (2006) *J Immunotoxicol*;34:227-44.
[43] Baken K.A., Arkusz J.,Pennings J.L., Vandebriel R.J., van Loveren H. (2007) *Toxicology*;2371-3:35-48.
[44] Grinwis G.C., Vethaak A.D., Wester P.W., Vos J.G. (2000) *Toxicol Lett*;112-113:289-301.
[45] Lewtas J. (1988) *Fundam Appl Toxicol*;104:571-89.
[46] Elespuru R.K.,Agarwal R.,Atrakchi A.H., Bigger C.A.,Heflich R.H.,Jagannath D.R.,Levy D.D.,Moore M.M., Ouyang Y., Robison T.W., Sotomayor R.E., Cimino M.C., Dearfield K.L. (2009) *Toxicol Sci*;1092:172-9.
[47] Kirkland D.,Aardema M., Henderson L., Müller L. (2005) *Mutat Res*;5841-2:1-256.
[48] Purves D.,Harvey C., Tweats D.,Lumley C.E. (1995) *Mutagenesis*;104:297-312.
[49] Ritter D., Knebel J. (2009) *Toxicol In Vitro*.
[50] González M., Soloneski S., Reigosa M.A., Larramendy M.L. (2005) *Toxicol In Vitro*;192:289-97.
[51] Molinari G.,Soloneski S., Reigosa M.A.,Larramendy M.L. (2009) *J Hazard Mater*;1651-3:1074-82.
[52] Thybaud V.,Aardema M.,Casciano D.,Dellarco V, Embry M.R.,Gollapudi B.B.,Hayashi M.,Holsapple M.P.,Jacobson-Kram D.,Kasper P.,MacGregor J.T., Rees R. (2007) *Mutat Res*;6332:67-79.
[53] Pilili J.P.,González N.V.,Molinari G.,Reigosa M.A.,Soloneski S.,Larramendy M.L.2009,Biologicals.
[54] Dekant W.,Melching-Kollmuß S.,Kalberlah F. (2009) *Regul Toxicol Pharmacol*.
[55] Fang H.,Harris S.C.,Su Z.,Chen M.,Qian F.,Shi L.,Perkins R.,Tong W. (2009) *Methods Mol Biol*;563:379-98.
[56] Watanabe H.,Takahashi E.,Nakamura Y.,Oda S.,Tatarazako N., Iguchi T. (2007) *Environ Toxicol Chem;*264:669-76.
[57] Neumann N.F.,Galvez F. (2002) *Biotechnol Adv;*205-6:391-419.
[58] Rooney J.P.,Patil A.,Zappala M.R.,Conklin D.S.,Cunningham R.P.,Begley T.J. (2008) *DNA Repair Amst;*711:1855-68.
[59] Maurer M.,Molidor R.,Sturn A.,Hartler J.,Hackl H.,Stocker G., Prokesch A.,Scheideler M.,Trajanoski Z. (2005) *BMC Bioinformatics*;6:101.
[60] Mortensen A.S.,Arukwe A. (2007) *Chem Res Toxicol*;203:474-88.
[61] Fan X.,Shi L.,Fang H.,Harris S.,Perkins R.,Tong W. (2009) *BMC Proc*;3 Suppl 2:S4.

[62] Foster W.R.,Chen S.J.,He A.,Truong A.,Bhaskaran V.,Nelson D.M.,Dambach D.M.,Lehman-McKeeman L.D.,Car B.D. (2007) *Toxicol Pathol*;355:621-35.
[63] Boess F.,Durr E.,Schaub N.,Haiker M.,Albertini S.,Suter L.2007,Toxicol In Vitro;217:1276-86.
[64] Boverhof D.R.,Burgoon L.D.,Tashiro C.,Sharratt B.,Chittim B.,Harkema J.R.,Mendrick D.L., Zacharewski T.R. (2006) *Toxicol Sci*;942:398-416.
[65] Kopec A.K.,Boverhof D.R.,Burgoon L.D.,Ibrahim-Aibo D.,Harkema J.R.,Tashiro C.,Chittim B.,Zacharewski T.R. (2008) *Toxicol Sci*;1021:61-75.
[66] Marsman D. (1995) *Toxic Rep Ser*;30:1-G5.
[67] Boedigheimer M.J.,Wolfinger R.D.,Bass M.B.,Bushel P.R.,Chou J.W.,Cooper M.,Corton J.C.,Fostel J.,Hester S.,Lee J.S.,Liu F.,Liu J.,Qian H.R.,Quackenbush J.,Pettit S.,Thompson K.L. (2008) *BMC Genomics*;9:285.
[68] Anderson N.,Meier T.,Borlak J. (2009)*Toxicol Sci.*
[69] Decristofaro M.F.,Daniels K.K.(2008) *Methods Mol Biol*;460:185-94.
[70] Singh G.,Driever P.H.,Sander J.W. (2005) *Brain*;128Pt 1:7-17.
[71] Le Mée S.,Chaminade F.,Delaporte C.,Markovits J.,Saucier J.M.,Jacquemin-Sablon A.(2000) *Mol Pharmacol*;584:709-18.
[72] Dardes R.C.,O'Regan R.M.,Gajdos C.,Robinson S.P.,Bentrem D.,De Los Reyes A.,Jordan V.C. (2002) *Clin Cancer Res*;86:1995-2001.
[73] Naranmandura H.,Ogra Y.,Iwata K.,Lee J.,Suzuki K.T.,Weinfeld M.,Le X.C. (2009) *Toxicol Appl Pharmacol*;2382:133-40.
[74] Davtyan T.K., Mkhitaryan L.M., Gabrielyan E.S. (2009) *Curr Pharm Des*;1511:1172-86.
[75] O'Regan R.M., England G.M., Macgregor J.I., Yao K.A., Muenzner H.D., Takei H., Jordan V.C. (1998) *Breast Cancer*;53:211-7.
[76] Dardes R.C., Schafer J.M., Pearce S.T., Osipo C., Chen B., Jordan V.C. (2002) *Gynecol Oncol;*853:498-506.
[77] Hatori Y., Sharma R.P., Warren R.P. (1991) *Immunopharmacology*;222:127-36.
[78] Martin R.,Rose D.,Yu K.,Barros S. (2006) *Pharmacogenomics*;77:1003-16.
[79] Hayashi P.H. (2009) Semin Liver Dis;294:348-56.
[80] McMillian M., Nie A., Parker J.B., Leone A., Kemmerer M., Bryant S., Herlich J., Yieh L., Bittner A., Liu X., Wan J., Johnson M.D., Lord P. (2005) *Toxicol Appl Pharmacol;*2072 Suppl:171-8.
[81] Roh J.Y., Sim S.J., Yi J., Park K., Chung K.H., Ryu D.Y., Choi J. (2009) *Environ Sci Technol*;4310:3933-40.
[82] Kondo C., Minowa Y., Uehara T., Okuno Y., Nakatsu N., Ono A., Maruyama T., Kato I., Yamate J., Yamada H., Ohno Y., Urushidani T. (2009) *Toxicology;*2651-2:15-26.
[83] Lee Y.S., Yoon S., Yoon H.J., Lee K., Yoon H.K., Lee J.H., Song C.W. (2009) *Toxicol Lett;*1893:191-9.
[84] Goetz A.K., Dix D.J. (2009) *Toxicol Appl Pharmacol*;2381:80-9.
[85] Nelson T.J., Behfar A., Terzic A. (2008) *Clin Transl Sci*;12:168-171.
[86] Erdo F., Buschmann I.R. (2007) *Orv Hetil*;14814:633-42.
[87] Casalini P., Caldera M., Canevari S., Ménard S., Mezzanzanica D., Tosi E., Gadina M., Colnaghi M.I. (1993) *Cancer Immunol Immunother*;371:54-60.
[88] Da Silva V.F., Feeley M., Raaphorst G.P.(1991)*J Neurooncol*;111:37-41.
[89] Pestronk A., Drachman D.B., Teoh R., Adams R.N.(1983) *Ann Neurol.*;142:235-41.
[90] Peachey J.E., Annis A.(1984) *Psychiatr Clin North Am.*;74:745-56.
[91] Carrera C.J., Terai C., Lotz M., Curd J.G., Piro L.D., Beutler E., Carson D.A.(1990) *J Clin Invest;*865:1480-8.
[92] Lenz D., Barten M.J., Hiller S., Tárnok A., Sack U. (2005) Cytometry A.;642:110-4.
[93] Nelson T.J., Behfar A., Yamada S., Martinez-Fernandez A., Terzic A. (2009) *Clin Transl Sci*;23:222-227.
[94] Klein C.A. (2003) *Verh Dtsch Ges Pathol;*87:158-64.
[95] Frilling A., Li J., Malamutmann E., Schmid K.W., Bockisch A., Broelsch C.E. (2009) *Br J Surg*;962:175-84.
[96] Van Allen C.M.(1925) *J Exp Med*;416:691-705.
[97] Thomas E. Wyatt, M.D.(1938) *Ann Surg*;1072:260-9.
[98] Baserga R., Putong P. B., Tyler S. and Wartman W. B. (1960) *Br J Cancer*;142: 173–185.
[99] Ozan E., Oztekin O., Alacacioglu A., Aykas A., Postaci H., Adibelli Z. (2009) *Diagn Interv Radiol*,1861-08.2.
[100] Ian F. Robey, Brenda K. Baggett, Nathaniel D. Kirkpatrick, Denise J. Roe, Julie Dosescu,Bonnie F. Sloane, Arig Ibrahim Hashim, David L. Morse, Natarajan Raghun,Robert A. Gatenby, and Robert J. Gillies (2009) *Cancer Res;*696:2260–8.
[101] Lee Y.S., Yoon S., Yoon H.J., Lee K., Yoon H.K., Lee J.H., Song C.W. (2009) *Toxicol Lett;*1893:191-9.

[102] Agathanggelou A., Bièche I., Ahmed-Choudhury J., Nicke B., Dammann R., Baksh S., Gao B., Minna J.D., Downward J., Maher E.R., Latif F. (2003) *Cancer Res*;6317:5344-51.
[103] Jankiewicz M., Groner B., Desrivières S. (2006) *Mol Endocrinol*;2010:2369-81.
[104] Yang Y., Wang H.C., Sun X.H. (2008) *BMC Immunol*;9:20.
[105] Chao J.I., Kuo P.C., Hsu T.S. (2004) *J Biol Chem*;27919:20267-76.
[106] Li J., Viallet J., Haura E.B. (2008) *Cancer Chemother Pharmacol*;613:525-34
[107] Chaudhary J., Johnson J., Kim G., Skinner M.K. (2001) *Endocrinology;*1425:1727-36.
[108] Kandasamy K., Srivastava R.K. (2002) *Cancer Res*;6217:4929-37.