[S1-2] [10/19/2001(Fri) 11:00-11:30 / Hall A]

Current Trend of Advanced Methods in Cellular and Molecular Toxicology

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I. Introduction

The detection and the regulation of man-made synthetic chemicals are subjects of great concern in administrative authorities because of its close correlation between environmental contamination and human health. It has been widely assumed that mutation represents at least one step in carcinogenesis. The evidence supporting this idea is that the majority of mutagens are carcinogens (McCann *et al.*, 1975) and, for at least some compounds, mutagenic potency is closely correlated with carcinogenic potency (Meselson and Russel, 1977). The establishment of toxicity that may pose a genetic hazard in our environment is subjects of great concern at present. Since the tens of thousands of man-made chemicals that have been introduced into the environment in the last few decades must also be tested for their damaging effect on DNA, the agents that cause this damage must be identified. Generally, the carcinogenicity including genotoxicity is one of the potential toxicity that may consider for the human health. Moreover, mutagens and certain non-mutagenic carcinogens have also been found to induce chromosomal rearrangement (Zimmermann, 1971) which may affect carcinogenesis by altering gene expression, perhaps by allowing the activation or inactivation of cellular cancer genes (Radman *et al.*, 1982). Several assay systems with rapidity and reliability have been introduced for this purpose, such as reversion test with bacterial gene mutation (Ames et al, 1973, 1975; Maron and Ames, 1983), chromosomal aberration assay with mammalian cells (Ishidate and Odashima, 1977), and micronucleus assay with rodents (Hayashi et al, 1992; Schmid, 1975). These assay systems are now well used to evaluate the genotoxicity of chemicals, and also frequently adopted as methods for an index of genotoxicity worldwide. Furthermore, it is well applied as a screening probe for the detection of possible carcinogenic substances in our environment.

Practically, several short term methods have been applied (Ames *et al.*, 1973;

Maron and Ames, 1983; Mersch-Sundermann *et al.*, 1991) for predicting the carcinogenicity of chemicals and also been introduced to the evaluation of genotoxicity (Ishidate and Odashima, 1977; Matsuoka *et al.*, 1979; Radman *et al*, 1982; Hayashi *et al*, 1982, 1990, 1992; Ryu *et al.*, 1993a, 1994a,b, 1996a,b, 1998b,d, 2001b) and of antimutagenicity (Sato *et al.*, 1991; Ryu *et al.*, 1993b, 2001c). Cytogenetic studies on mammalian cells *in vivo* (Schmid, 1975; Hayashi *et al.*, 1982, 1990, 1992, 1994a; Heo *et al.*, 1997) as well as *in vitro* (Ishidate and Odashima, 1977; Matsuoka *et al.*, 1979) have also been widely used as a screening method for DNA-attacking substances.

However, these kinds of toxicity evaluation tools cannot elucidate the mode and/or mechanism of actions of chemicals, especially carcinogens and mutagens. Recently developed transgenic mutagenesis assay system is subjected for this purpose because this assay system may provide a powerful tool to predict the mutation spectrum induced in cancer-related genes more accurately. Moreover, many scientists try to develope more precise, convenient and sensitive techniques for the detection of DNA damages as an index of carcinogenicity.

Recently, several new methods for the detection of genetic damages *in vitro* and *in vivo* were introduced according to the rapid progress in toxicology combined with cellular and molecular biology. Among these methods, the single cell gel electrophoresis (comet assay) which can be detected DNA damages in cell level (Mckelvey-Martin *et al.*, 1993; Singh *et al.*, 1994; Ryu *et al.*, 1997; Tice *et al.*, 2000), mouse lymphoma thymidine kinase gene assay (Clive *et al.*, 1983; Sawyer *et al.*, 1985; Ryu *et al.*, 1999a), FISH (fluorescence *in situ* hybridization) (Hayashi *et al.*, 1994b), PRINS (primed *in situ* hybridization) (Abbo *et al.*, 1993) and transgenic animal and cell line model as a parameter of *lac I* (Big Blue) (Kohler *et al.*, 1991; Ryu *et al.*, 1998a,c, 1999a, 2000, 2001d) or *lac Z* (Muta Mouse) (Suzuki *et al.*, 1993) gene mutation are newly introduced based on cellular and molecular toxicological approaches. Also, *in vivo* supravital micronucleus assay with peripheral reticulocytes by using acridine orange fluorescent staining (Hayashi *et al.*, 1990, 1992; Ryu *et al.*, 1998b) was introduced instead of mouse bone marrow micronucleus assay. In this presentation, I will introduce recent advanced toxicological methods to evaluate and to elucidate the mechanism of genetic toxicity and/or carcinogenesis.

II. Single Cell Gel Electrophoresis (Comet) assay

As one of the mechanisms of carcinogenicity, induction of DNA damage was ascertained by a comet assay, which is widely used for the detection and measurement

of DNA strand breaks. Since Ostling and Johanson (1984) introduced microelectrophoretic technique, Singh *et al.* (1988) have modified and improved the microgel electrophoresis technique to evaluate DNA damage in single cells under alkaline conditions. The single cell gel electrophoresis (SCGE, comet, microgel electrophoresis) assay is rapid, sensitive, visual and simple technique to quantify DNA strand breaks in individual cells. It can be noticed that there are at least 3 major basic protocols: the first of Ostling and Johanson (1984), the second of Singh et al. (1988, 1990, 1991a,b, 1994, 1995a,b) and the one of Olive et al. (1993, 1994). Each protocol follows the principle of embedding cells in agarose, lysing the cytoplasmic material and exposing the remaining cell nuclei to a weak electric field. Large differences can be found in the physical and chemical conditions of each version of the method.

If the agent can cause the strand breakage, we can see the extent of tail from the head (nucleus) like comet with stainning of fluorescent dyes such as ethidium bromide, acridine orange and propidium iodide etc. However, there are some variations in procedure and conditions from laboratory to laboratory and a kind of cells used. So, to harmonize this variations in comet assay, International Workshop on Genotoxicity Test Procedure (IWGTP) was held at Washington D.C. on March, 1999 by Environmental Mutagen Society supported with OECD. Our laboratory (Ryu et al, 1999c, 2001a) also involved in this harmonization and published as preliminary form for OECD guideline with Tice *et al.* (2000). Comet assay is useful in various fields such as radiation, human monitoring in work place and toxicity evaluation of some chemicals etc. General reviews on this technique have been published by Tice et al. (1991, 2000) and Fairbairn et al. (1995, 1996).

II-1 : Cell culture and viability test

The mouse lymphoma cell line L5178Y (*tk*+/- 3.7.2c) was used for the experiment. Cells were cultivated in 90% RPMI-1640 (Life Technologies, MD, USA) with 1 mM sodium pyruvate, 0.1% pluronic supplemented with 10% heat-inactivated horse serum and antibiotics in a humidified incubator at 37 0C with 5% CO2. For the determination of cell viability, about 106 cells were treated for 2 hr with test chemicals. BaP was used in combination with S9 mixture. After the staining of 0.4% trypan blue (Life Technologies, MD, U.S.A), the total number of cells and the number of unstained cells were counted in five of the major sections of a hemocytometer. The average number of cells per section was calculated. Cell viability of treated cultures was related to controls that were treated with the solvent and S9 mixture. All experiments were repeated twice in an independent experiment.

II-2 : Preparation of agarose coated glass slide

For the comet assay, 8 x 105 cells were seeded into 12 wells plate (Falcon 3043) and then treated with test compounds. In the experiments with metabolic activation, parallel cultures were performed in the absence or presence of S9 mixture. After 2 min, cells were centrifuged for 5 min at 100 x g (about 1,200 rpm), and gently resuspended with PBS and 100 $\mu\ell$ of the cell suspension was immediately used for the test. Cells were mixed with 0.1 ml of 1% low melting point agarose (LMPA, Gibco BRL, Life Technologies, Inc., MD, USA) and added to fully frosted slide (Cat. No., 12-544-5, Fisher Scientific, PA, USA) which had been covered with a bottom layer of 100 $\mu\ell$ of 1% normal melting agarose (Amresco, OH, USA). The cell suspension was immediately covered with cover glass and the slides were then kept at 40C for 5 min to allow solidification. After removing the cover glass gently, the slides were covered with a third layer of 100 $\mu\ell$ of 0.5% LMPA by using a cover glass and then the slide were kept again at 40C for 5 min.

II-3 : Alkaline unwinding and alkaline electrophoresis

The procedure follows the method described by Singh *et al.*, (1988) with minor modification. The cells embedded in the agarose on slides were lysed for 1.5 hr in reaction mixture of 2.5 M NaCl, 0.1 M Na2-EDTA, 10 mM Tris-HCl (pH 10), 1% Triton X-100 at 40C. Slides were then placed in 0.3 M NaOH containing 1 mM Na2-EDTA (approximately pH 13) for 20 min to unwind DNA before electrophoresis. Electrophoresis was conducted at 25 V (about 1 V/cm across the gels) and approximately 300 mA for 20 min at 40C. All of the steps described above were conducted under yellow light or in the dark to prevent additional DNA damage.

II-4 : Evaluation and Statistics of DNA damage

After the electrophoresis, the slides were washed gently to remove alkali and detergents which would interfere with ethidium bromide staining, by placing horizontally and flooding them three times slowly with 0.4 M Tris (pH 7.5) for 5 min. The slides were stained by 50 $\mu\ell$ of ethidium bromide in distilled water solution on each slide, and then covering the slide with a cover glass. Image of 100 randomly selected cells (50 cells from each of two replicate slides) was analysed from each sample. All experiments were repeated in an independent test. Measurement was made by image analysis with Komet 3.1 (Kinetic Imaging Limited, Liverpool, UK) system, determining the mean tail moment (percentage of DNA in the tail times tail length) of

the 50 cells. Differences between the control and the other values were tested for significance using one way of analysis of variance (ANOVA).

III. Mouse lymphoma thymidine kinase (tk)+/- gene assay (MOLY)

MOLY was performed with L5178Y tk+/- mouse lymphoma cells as described by Clements (1994), with minor modification (Clive et al., 1983, 1995, Sawyer et al., 1985; Garriott et al., 1995; Oberly and Garriott, 1996; Sofuni et al., 1996, Ryu et al, 1999b). The cytotoxicity of chemical was determined by relative survival (RS) after 3 hr treatment at concentrations up to 5000 g/ml with and without S-9 mixture. The highest concentration chosen was one with a 10-20% RS. This experiment consisted of one solvent control, one positive control and at least three test chemical concentrations in duplicate cultures. Cultures were exposed to the test chemical for 3 hr, then cultured for 2 days before plating in 96-well microtiter plates at 2000 cells/well with trifluorothymidine for mutant selection and at 1.6 cells/well for cell viability. The number of wells containing colonies was counted on day 12 after plating, and large and small colonies were scored. Mutation frequencies were analyzed by the statistical package, Mutant V2.31 program (Hazleton, England) in accordance with the UKEMS guidelines. The acceptable ranges of mean absolute plating efficiency (PE) for solvent control were 60-140% for survival (PE0) and 70-130% for viability (PE2), based on the consensus agreements (Clive et al., 1995).

IV. Supravital staining *in vivo* micronucleus assay with peripheral reticulocytes

The micronucleus (MN) assay *in vivo* is a method devised primarily for screening chemicals for chromosome-breaking effects. In the monitoring of chromosome breakage, the test is at least as sensitive as the metaphase method; in addition it includes effects on the spindle apparatus. This properties render the assay highly suitable for routine toxicological screening. The method is based on the following principles and observations : in anaphase, acentric chromatid and chromosome fragments lag behind when the centric elements move towards the spindle poles. After telophase the undamaged chromosomes, as well as the centric fragments, give rise to regular daughter nuclei. The lagging elements are included in the daughter cells, too, but a considerable proportion is transformed into one or several secondary nuclei which are, as a rule, much smaller than the principal nucleus and are therefore called micronuclei.

The MN assay using peripheral blood erythrocytes was introduced by MacGregor et al. (1980) and developed by Hayashi et al. (1990, 1994b) into more simple and

convenient method compared to conventional bone marrow assay by the introduction of supravital acridine orange staining. Giemsa staining has been routinely used for the MN assay. The Giemsa method, however, has some disadvantages because not only MNs but also some cell inclusions containing RNA and other acidic materials are stained dark blue by Giemsa; it is occasionally difficult to identify MNs from these inclusions. Aciridine Orange (AO) metachromatic fluorochrome discriminates between DNA and RNA by green and red fluorescence, respectively. The method provides advantages that high-quality specimens can be easily obtained without special skill, and can be reduced intra- and inter-laboratory variation.

When this method is combined with other toxicological studies, chromosomal damage could be assessed together with other toxicological endpoints.

V. *In vitro* cytokinesis-block micronucleus assay

The study of DNA damage at the chromosome level is an essential part of genetic toxicology because chromosomal mutation is an important event in carcinogenesis. The micronucleus assays have emerged as one of the preferred methods for assessing chromosome damage because they enable both chromosome loss and chromosome breakage to be measured reliably (Heddle et al., 1983). Because micronuclei can only be expressed in cells that complete nuclear division a special method was developed that identifies such cells by their binucleate appearance when blocked from performing cytokinesis by cytochalasin-B, a microfilament-assembly inhibitor. The cytokinesis-block micronucleus (CBMN) assay allows better precision because the data obtained are not confounded by altered cell division kinetics caused by cytotoxicity of agents tested or sub-optimal cell culture conditions. The method is now applied to various cell types for population monitoring of genetic damage, screening of chemicals for genotoxic potential and for specific purposes such as the prediction of the radiosensitivity of tumours (Shibamoto et al., 1991) and the inter-individual variation in radiosensitivity. In its current basic form the CBMN assay can provide, using simple morphological criteria, the following measures of genotoxicity and cytotoxicity: chromosome breakage, chromosome loss, chromosome rearrangement (nucleoplasmic bridges), cell division inhibition, necrosis and apoptosis (Fenech et al., 1999). The cytosine-arabinoside modification of the CBMN assay allows for measurement of excision repairable lesions (Fenech et al., 1994). The use of molecular probes enables chromosome loss to be distinguished from chromosome breakage and importantly non-disjunction in non-micronucleated binucleated cells can be efficiently measured (Zihno et al., 1994). The

in vitro CBMN technique, therefore, provides multiple and complementary measures of genotoxicity and cytotoxicity which can be achieved with relative ease within one system.

VI. Transgenic Mutagenesis assay system

Because mutagens often produce characteristic patterns of DNA sequence alterations (Dogliotti, 1996), called mutation spectrum, elucidation of mutation spectrum of specific carcinogen provides a basis for understanding of cancer etiology and action mechanism of chemical carcinogenesis (Gorelick, 1995).

Recently developed transgenic mutagenesis assay system including Big Blue mutagenesis system is a useful and powerful tool to evaluate the genotoxicity of chemicals, and it also provides a window of carcinogenesis and mutagenesis mechanisms of chemicals based on information such as mutation pattern, frequency, and location in sequence context of the *lacI* target gene (Gorelick, 1995). The *lacI* transgenic Big Blue Rat2 fibroblast cell line carries over 40 copies of lambda shuttle vector (Dycaico et al, 1994) containing *lacI* gene as a target (Lundberg et al, 1993; Heddle and Tao, 1995; Summers et al, 1989). The *lacI* gene, as a mutational target, is very useful for the study of the mutational characteristics of a carcinogen for several reasons. First, the relatively small size (1,080 bp of coding region) of *lacI* gene facilitates sequence analysis. Second, the expression of repressor protein permits a rapid colorimetric assay to screen for mutations. In addition, it is possible to make use of the large historical database in subsequent comparisons, allowing us to elucidate the underlying mechanisms leading to mutations (Kohler et al, 1991). The mutations induced in the *lacI* gene can easily be quantified by mutant frequency (MF), and the precise mutation type and distribution can quickly be identified by direct sequencing. Moreover, considering that mutations in *lacI* gene induced by chemicals reflect the effects of mutagens on other endogenous genes such as proto-oncogenes and tumor suppressor genes, and that mutations occurred in these genes are the most common events in many types of human cancer (Kohler et al, 1991; Tao et al, 1993; Gossen et al, 1989), this assay may provide a powerful tool to predict the mutation spectrum induced in cancer-related genes more accurately. Our laboratory elucidated mutation spectrum of 4-nitroquinoline N-oxide (Ryu et al, 1998a,c, 1999a). And also, we reported the mutant frequency of atrazine with cytogenetic analysis using Big Blue Rat2 cells (2000).

VI-1 : Cell culture

The *lacI* transgenic Big Blue Rat2 fibroblast cell line was purchased from Stratagene (La Jolla, CA). This cell line is derived from a Rat2 embryonic fibroblast cell line (CRL 1764; ATCC, Rockville, MD) transfected with the Big Blue shuttle vector (lambda *lacI* / *lacZ* shuttle vector) and pSV2NEO plasmid, which provides an antibiotic selection marker. The shuttle vector, which has been integrated into the Rat2 genome at two sites (~60 copies/cell), is identical to that used for generating the Big Blue mouse. The *lacI* transgenic Big Blue Rat2 fibroblast cells were cultured as a monolayer in Dulbeccos modified Eagles medium (DMEM; Gibco BRL, NY) containing 50 units/ml penicillin, 50 mg/ml streptomycin, 200 mg/ml geneticin (G418), and 10% heat-inactivated defined fetal bovine serum (Gibco BRL, NY) at 37℃ in a humidified incubator containing 5% CO2 (Ryu et al, 1999a). Under these conditions, the average cell cycle time was ~19 h. Low passage (<5) cells were used in each experiment. Cells were treated at 30-40% confluence (~3.5x105 cells in a 25 cm2 flask). A solution of 0.25% trypsin-EDTA (Gibco BRL, NY) was used for subculture.

VI-2 : Transgenic mutagenesis assay

a : Isolation of genomic DNA. Genomic DNA was isolated from *lacI* transgenic Big Blue Rat2 fibroblast cells using the RecoverEase protocol (Stratagene, La Jolla, CA). In essence, cell pellets were thawed on ice, resuspended in 8 ml of ice-cold lysis buffer (8.20 g NaCl, 0.22 g KCl, 120 g sucrose, 0.30 g EDTA, 10 ml Triton X-100, 1.58 g Tris-HCl (pH 8.3)) and homogenized with a tissue grinder. The homogenate was filtered through a 100 ㎛ nylon mesh filter into 50 ml tubes and centrifuged at 1,100 x *g* for 12 min at 4℃. The supernatant was discarded and the tubes were swabbed with a cotton swab to remove residual supernatant. The pellet was incubated at 50℃ for 45 min in the presence of 70 ㎕ digestion buffer containing RNace-It ribonuclease cocktail (20㎕/ml digestion buffer; Stratagene, La Jolla, CA) and 70 ㎕ proteinase K solution. The contents of the tube were dialyzed for 48 h by placing the DNA extract on the wetted surface of a membrane floating on 10 mM Tris/1 mM EDTA buffer, pH 7.4. The viscous DNA was collected and stored at 4℃ until packaging.

b : Packaging and plating of DNA. After DNA concentrations were adjusted to 0.5 mg/ml, the genomic DNA was incubated with Transpack packaging extract (Stratagene, La Jolla, CA) to excise the lambda vector target and package it into a lambda head according to the Stratagene Big Blue instruction manual. The titer of the rescued phage was estimated by plating the packaged phage in serial dilutions. For plating, the volume of the phage equivalent to 15,000 plaque forming units was added to 2 ml of *Escherichia coli* SCS-8 cells (Stratagene, La Jolla, CA) in 10 mM MgSO4.

Pre-warmed Big Blue top agarose containing 1.5 mg/ml of X-gal (dissolved in dimethylformamide) was added, and the contents were poured onto 25 x 25 cm2 assay trays containing 250 ml Big Blue bottom agar (agar, casein peptone, yeast extract, MgSO4 and NaCl). Trays were incubated for 18 h at 37℃ and scored for blue mutant plaques. The total number of plaques in each plate was estimated by counting four sectors of 5 5 cm2 areas, averaging these numbers and multiplying by a scaling factor. Blue mutant plaques were counted and picked into individual tubes containing 0.5 ml SM buffer (5.8 g NaCl, 2.0 g MgSO47H2O, 50 ml 1 M Tris-HCl (pH 7.5) and 5 ml of 2% gelatin (W/V)) for further characterization. To confirm the mutant phenotype and for future use in DNA sequence analysis, all recovered putative mutant phages were diluted to 1:200 and replated on 100 mm plates with 3.5 ml of top agarose containing 1.5 mg/ml X-gal. The sectored plaques that were observed were also verified for their phenotype as previously specified and confirmed sectored plaques were scored separately. The *lacI* mutant frequency (MF) value was calculated by dividing the number of verified mutant plaques by the total number of plaques analyzed.

VI-3 : Mutation spectrum

The *lacI* gene was sequenced from *lacI* mutants of solvent and medium control cells (collectively considered as spontaneous mutants) and of cells exposed to chemical. Each mutant was purified by replating on a 100 mm plate at low density. From each plate, a single mutant plaque was isolated, cored, placed into 25 ㎕ deionized water, and then boiled for 5 min. The *lacI* gene was amplified by PCR in 100 ㎕ PCR reaction mixture containing 15 ㎕ of phage DNA, 2 ㎕ of each of forward position 53 to 37, (5-CCCGACACCATCGAATG-3) and reverse position 1,201 to 1,185, (5-ACCATTCCACACAACATAC-3) Big Blue PCR primers, 25 mM dNTPs and 5 U *Ex Taq* polymerase (TaKaRa Shuzo Co. Ltd, Japan). After the initial denaturation step at 95℃ for 5 min and extension step at 72℃ for 5 min, 30 cycles of amplification were performed as follows: denaturation at 95℃ for 90 s, annealing at 55℃ for 90 s, elongation at 72℃ for 150 s with a final extension at 72℃ for 10 min using thermocycler (Robocycler 40 temperature cycler, Stratagene, La Jolla, CA). The resulting 1,300 bp fragment was purified with a PCR purification kit (Inje Biotech, Seoul, Korea) according to the manufacturers instructions, and visualized on a 1% low melting point (LMP) agarose gel containing 1 mg/ml ethidium bromide. The PCR products were sequenced with an ABI PRISM dye terminator cycle sequencing ready reaction kit (Perkin-Elmer, CA) on an Applied Biosystem 373 DNA sequencer (Perkin-Elmer, CA). The purified DNA sample corresponding to approximately 200 ng was

added to 3.2 pmol/ml primer and terminator dyes. The sequencing reactions were carried out 25 cycles as follows: 96℃ for 30 s; 50℃ for 15 s; 60℃ for 4 min, according to the protocol established by Applied Biosystems.

VI-4 : Statistical analysis

Analysis of variance (ANOVA) test was performed to evaluate differences in *lacI* mutant frequency between treatment groups at the level of = 0.05. A Dunnetts multiple comparison test was used to compare each treatment group with untreated controls. Chi-square (2) test was used for statistical treatment of mutational spectra.

VII : Toxicogenomics

The recent completion of the human genome sequencing project and the push to finish the mouse genome have raised the stakes in science with predictions of disease cures, more effective and safer pharmaceuticals, and a greater understanding of environmental effects on human health. The impact of human genome projects on toxicological research is high, heralding the emerging technologies of toxicogenomics, proteomics, and bioinformatics (Lovett, 2000, Pollack, 2000) for the future use of these technologies and their impact on drug discovery, safety evaluation, elucidation of pathways of toxicity, and risk assessment. The utilization of these new technologies along with more established genetic approaches such as quantitative polymerase chain reaction (PCR), and the use of genetically altered animals will dramatically move the field of toxicology forward.

cDNA and oligonucleotide arrays and high-throughput 2-D electrophoresis systems have quickly emerged as the premier tools to enable genomewide analysis of gene expression at the RNA and protein level. These new technologies are heavily influencing drug discovery and preclinical safety in the biotechnology and pharmaceutical industry (Freeman, 2000). Toxicologists are also promoting genomic expression technologies as a superior alternative to traditional rodent bioassays to identify and assess the safety of chemicals and drug candidates for human safety (Afshari et al., 1999; Nuwaysir et al., 1999; Pennie et al., 2000). It is expected that gene expression profiling will identify mechanisms of action that underlie the potential toxicity of chemicals and drug candidates. Ultimately, toxicogenomics (the integration of genomics, bioinformatics, and toxicology) is expected to accelerate drug development and aid risk assessment. Recent experiments applied to cancer genetics have demonstrated the potential of gene expression profiling to accurately classify

disease phenotypes (Alizadeh et al., 2000; Bittner et al., 2000), thus lending hope that expression profiling may classify and thus predict phenotypes of toxicity. Despite these expectations, it is still uncertain how gene expression profiling experiments will ultimately contribute to our understanding of toxicity and allow us to realize the full potential of this new technology. Pennie et al. (2000) have also discussed the possibilities and caveats of gene expression profiling in the context of mechanistic and predictive toxicology and have addressed the certainty, biological relevance, and need for validation of microarray data.

There are both advantages and limitations to the use of gene array and proteomics technologies in toxicologic screening. The main advantage is a global approach to understanding the complex mechanisms involved in toxicology. Gene arrays have been costly and limited in availability, but the past year has shown a commitment by the scientific community to the general use and availability of gene arrays. Consequently, cost has been reduced by increased supply and demand. Furthermore, the availability and cost is substantially improving with many universities and research centers establishing genomic and proteomic facilities. Therefore, toxicogenomics and proteomics will certainly become generally used technologies in the near future.

VIII. References

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