have been reported. These methods treat less than 10 samples and fit into common CO_2 incubators. The radial transmission line (RTL) system [Moros et al., 1999] incorporates a self-contained environmental control function and regulates the temperature of the samples, though CO_2 is not provided. Approximately 20 samples can be placed in one RTL. A rectangular waveguide with slits, from which the electromagnetic field leaks and couples to the cell culture, has also been reported [Taki and Suzuki, 2002]. Among the open type RF exposure systems, a far field 2.45 GHz irradiation system [Harrison et al., 1985] in an anechoic chamber provides a 250 × 250 mm cell treatment area and has a self-contained coolant water circulation system to regulate the temperature of the samples. This system requires a large space because the distance between the RF exposure source (a horn antenna) and the samples is greater than 2 m. A cylindrical waveguide [Gajda et al., 2002] allows 1.9 GHz RF exposure of only one sample at the aperture of the waveguide because the distance between the RF exposure source and the sample is 3.2 mm. This system also regulates the sample temperature using a self-contained coolant water circulation system.

As mentioned above, an open type RF exposure system, where the samples are placed in the far field of an RF exposure source, allows many samples to be exposed simultaneously. To achieve simultaneous RF exposure of a large number of cells while minimizing the size of the RF exposure system, we proposed and developed an open type beam formed RF exposure-incubator in an anechoic chamber. In addition, to realize stable long term culture conditions, a self-contained environmental control unit separated from the RF exposure source supplied air at an appropriate temperature, CO_2 density, and humidity to the culture case. This study presents the design of the RF exposure source and the environmental control unit. Data on the specific absorption rate (SAR) distribution in the samples and on the cell culture including the temperature regulation in the culture rooms are also reported. The frequency of 2142.5 MHz, which corresponds to the middle frequency of the downlink band of the International Mobile Telecommunication 2000 (IMT-2000) cellular system, is used in the design of the RF exposure source and in the SAR evaluation.

MATERIALS AND METHODS

System Design

Two identical RF exposure-incubators, one for RF exposure and the other for sham exposure, are established in separate anechoic chambers. A schematic diagram depicting the electrical design and the air circulation of the system is shown in Figure 1A. The RF signal, which is fed from a signal generator (E4437B, Agilent Technologies, Palo Alto, CA) and amplified by a 20 W amplifier (AR Series 2-3 GHz, Comtech PST Corp., Melville, NY), is fed to either RF exposure source located in the respective anechoic chamber. The selection of RF exposure or sham exposure is actuated by mechanical switching in a dummy box. Both the forward and reflected powers are monitored using a 30 dB bidirectional coupler (440964, Microwave Corporation, Cheshire, CT), two power sensors (8481D, Agilent Technologies), and a dual channel power meter (E4419B, Agilent Technologies). All of the equipment except for the RF exposure-incubators is set outside the anechoic chambers in an area called the machine room. Figure 1B is a photograph of the RF exposure-incubator in the anechoic chamber. Customized software on a PC enables control of the frequency, modulation, power level, and exposure time of the RF signal. The PC also records the monitored forward and reflected powers, and the temperature in the culture cases (as described in the Environmental Control Unit).

RF Exposure Source

As shown in Figures 1A and B, the RF exposure source comprising a horn antenna and a dielectric lens radiates RF upwards. The horn antenna, which has an aperture of 187 × 146 mm, is facing upwards, and the dielectric lens, which has a diameter of 430 mm and a relative permittivity of 2.3, is constructed of high density polyethylene and is placed above the antenna. The RF exposure source operates at 2142.5 MHz. The distance between the aperture of the horn antenna and the center of the dielectric lens is 450 mm. The dielectric lens allows RF energy to be focused in the direction of the main beam of the horn antenna. The exposure table constructed of polyester, on which the culture case is set, is 500 mm above the dielectric lens. The height of the exposure table is 1100 mm. Except for the horn antenna and RF connectors, the RF exposure source is constructed using materials of low relative permittivity (less than 5).

Environmental Control Unit

As shown in Figures 1A and B, the culture case made of acrylic material has a two layer structure, that is, the atmosphere in the culture room and that in the air jacket are separated when the front door of the culture case is closed. The main unit, which is located outside the anechoic chambers, provides identical air through sealed ducts at the appropriate temperature (37 °C), CO_2 density (approximately 5%), and humidity (more

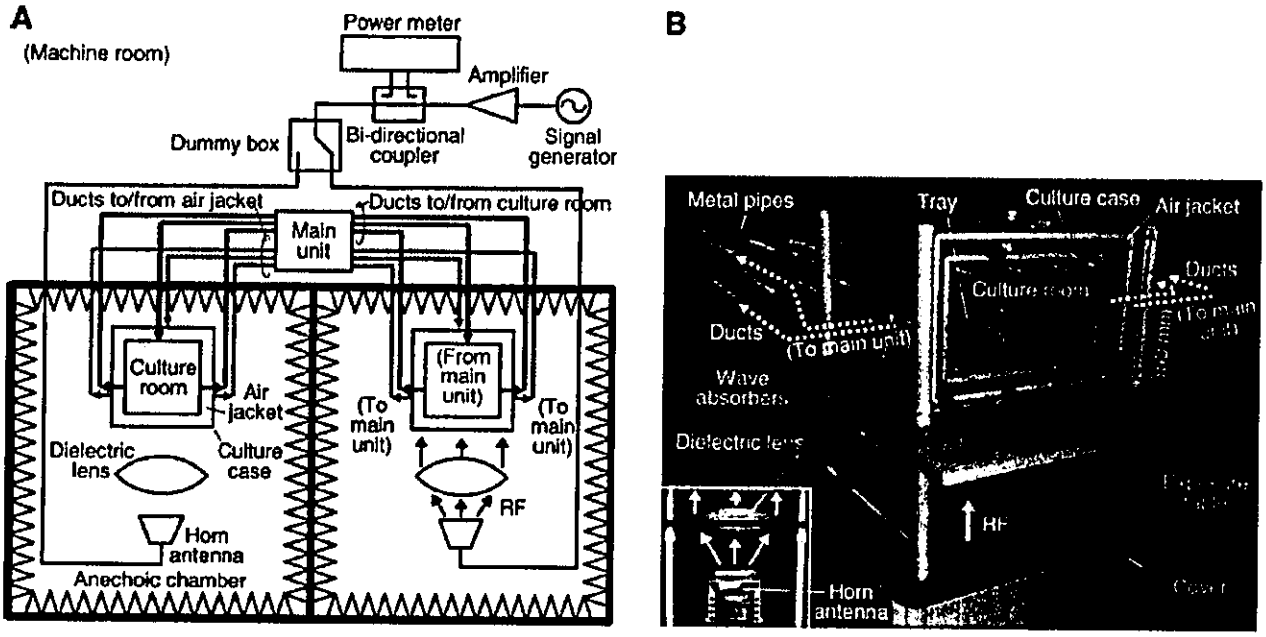

Fig. 1. RF exposure system for in vitro experiments. Arrows from the horn antenna indicate the direction in which the RF travels. **A**: Schematic diagram of the electrical design and the air circulation in the RF exposure system. In this figure, the system on the right is for RF exposure and that on the left is for sham exposure. **B**: Photograph of RF exposure-incubator employing a horn antenna, a dielectric lens and a culture case in an anechoic chamber. Dotted lines indicate the ducts for air circulation. Photograph at the lower left illustrates the inside of the covers of the RF exposure source.

than 90%) for cell culture in the two culture rooms. Warmer air (approximately 40 °C) is circulated in the air jackets through another duct to keep the temperature in the culture room stable. The dimensions of the culture room are 250 × 400 × 400 mm (height × width × depth) and those of the air jacket are 350 × 500 × 500 mm. A tray composed of vinyl chloride plastic is placed at the height of 100 mm from the bottom of the culture room to hold the culture dishes. The tray has a usable area of 340 × 340 mm corresponding to a 7 × 7 array of 35 mm diameter Petri dishes. Markings on the tray aid in the placement of the Petri dishes in the same location. The sensing element of a fiberoptic thermometer (AMOTH FL-2000 and FS100-*M, Anritsu Meter Co., Ltd, Tokyo, Japan), which is unaffected by RF, is inserted in the culture room to monitor the temperature. The accuracy of temperature measurement is 0.1 °C.

Anechoic Chamber

To achieve a sufficient degree of isolation between the two culture cases in terms of RF exposure, the RF exposure incubators are placed in separate anechoic chambers. The dimensions of the anechoic chamber are 2.7 × 2.6 × 3.95 m. The ceiling and four side walls including the entrance door are covered with RF absorbers. The shield panels and RF absorbers have an attenuation of greater than 80 dB above 800 MHz and a reflection loss of greater than 40 dB above 2 GHz, respectively. The wall facing the machine room has 12 circular metal pipes, as illustrated in Figure 1B, to which the ducts for air circulation in the culture case (culture room and air jacket) are connected. No RF energy leaks outside the anechoic chamber because the lowest cut-off frequency of the circular metal pipes, which have an inner diameter of 41.6 mm and a length of 220 mm, is 4.2 GHz (for TE_{11} mode). The anechoic chambers are constructed in a clean room (class 10 000) and the temperature is maintained at 22.5 ± 0.5 °C.

SAR Dosimetry

In our studies, adherent cells in a culture fluid with a height of 3 mm are cultivated in 35 mm diameter Petri dishes (353001 FALCON™, Becton, Dickinson and Company, Franklin Lakes, NJ). Therefore, the RF exposure level of the cells is determined as the SAR of the culture fluid at the bottom of the dish. The combination of an electric field measurement technique and the Finite Difference Time Domain (FDTD) [Taflove and Brodwin, 1975a] calculation method was used to assess the SAR distribution in the culture fluid. At 2142.5 MHz, the measurement results using the coaxial probe

(85070C, Agilent Technologies) and the vector network analyzer (8722ES, Agilent Technologies) indicated that the culture fluid had a relative permittivity of 76.4 and a conductivity of 2.5 S/m. The Petri dishes had a relative permittivity of 2.3.

The electric field distribution at the height that the Petri dishes were placed without a culture case was measured using an isotropic electric field probe constructed with three orthogonal 4 mm dipoles (ER3DV6, Schmid & Partners Engineering AG, Zürich, Switzerland) [Schmid et al., 1996], which was calibrated in air. This probe scanned in a 10 mm grid using a robot for industrial use (RX-90, Stäubli AG, Horgen, Switzerland).

It was impossible to measure the SAR in the 3 mm high culture fluid because of the size of the electric field probe. Therefore, the FDTD calculation method was used to evaluate the SAR of the culture fluid at the bottom of the Petri dishes used in the in vitro experiments. To confirm the effectiveness of the FDTD calculation, the SAR distribution in the culture fluid for a large culture dish (135 mm diameter and the fluid height of 18 mm) was measured in 1 mm steps in depth and on a 5 mm grid in the horizontal plane using the same electric field probe, which was calibrated in a liquid having similar permittivity and conductivity to those of the culture fluid used in our in vitro experiments, and scanning facility as described above. The data were compared to the calculated results using the FDTD calculation method assuming that the plane wave was incident from below and the amplitude was the same as the average electric field obtained by the measurement. The voxel size in the vertical direction was 0.5 mm, and that in the horizontal direction was 1.5 mm. The absorbing boundary condition of Mur's second approximation was assumed.

After the effectiveness of the FDTD calculation method was validated, the same calculation algorithm also gave the mean SAR and the SAR distribution in the culture fluid at the bottom of the 49 35 mm diameter Petri dishes used in the in vitro experiments filled with 3 mm high culture fluid. The effect of the culture case was considered negligible because it was constructed from low permittivity materials.

Cell Culture

The temperatures in the two culture rooms were measured using fiberoptic thermometers. The sensing elements of the thermometers were located at the center of each tray in the culture rooms. It was confirmed that the temperature measured using the fiberoptic thermometer was stable within the range of ± 0.1 °C even if it was placed in an E field of 100 V/m.

The proliferation of the H4 cell line in the RF exposure-incubators was compared to that in a common CO_2 incubator (BNA-121D, ESPEC CORP., Osaka, Japan). The H4 line of a 37 year old male's human neuroglioma cells obtained from American Type Culture Collection (ATCC; Rockville, MD) was maintained in Dulbecco's Modified Eagle's Medium (high glucose) containing 10% fetal bovine serum (DMEM; GIBCO BRL, Grand Island, NY). Twenty-five (5×5 array) 35 mm diameter Petri dishes filled with 3 mm high culture fluid, which contained 3.33×10^4 cells/ml, were placed in each self-contained culture case and a common CO_2 incubator. (The SAR dosimetry results indicated that SAR uniformity for the inner 25 Petri dishes was better than that for the total 49 Petri dishes when the 49 Petri dishes were simultaneously exposed. Therefore, only 25 Petri dishes containing the cells were evaluated in the cell culture test.) At the start of the cell culture test, almost the same number of cells was seeded in the two RF exposure-incubators and the common CO_2 incubator. Both of the RF exposure-incubators were sham exposed. To determine the number of viable cells, cell counting and/or measurement of the level of adenosine triphosphate (ATP) present were performed after 72 h. Cells were counted using a hemocytometer and the ATP level was measured using a luminescent cell viability assay system (CellTiter-Glo™, Promega Corporation, Madison, WI).

RESULTS AND DISCUSSION

SAR Dosimetry

The deviation of the electric field strength at the height that the Petri dishes were placed was ± 1.5 dB at 2142.5 MHz in the exposure area (300×300 mm) without the culture case. For comparison, it was ± 3 dB when the dielectric lens was removed. In addition, the electric field strength measurement with and without the dielectric lens at the center of the exposure area as mentioned above revealed that the gain of the dielectric lens was 7 dB.

The SAR distribution measured in the culture fluid for a large culture dish (135 mm diameter and the fluid height of 18 mm) as shown in Figure 2A and that obtained using the FDTD calculation method were compared while the culture case was not present. Figure 2B illustrates both of the SAR distributions, which are normalized to that obtained using the FDTD calculation method at the bottom of the culture dish, along the central vertical axis. SAR measurement in the culture fluid lower than 5 mm from the bottom of the culture dish could not be performed because the

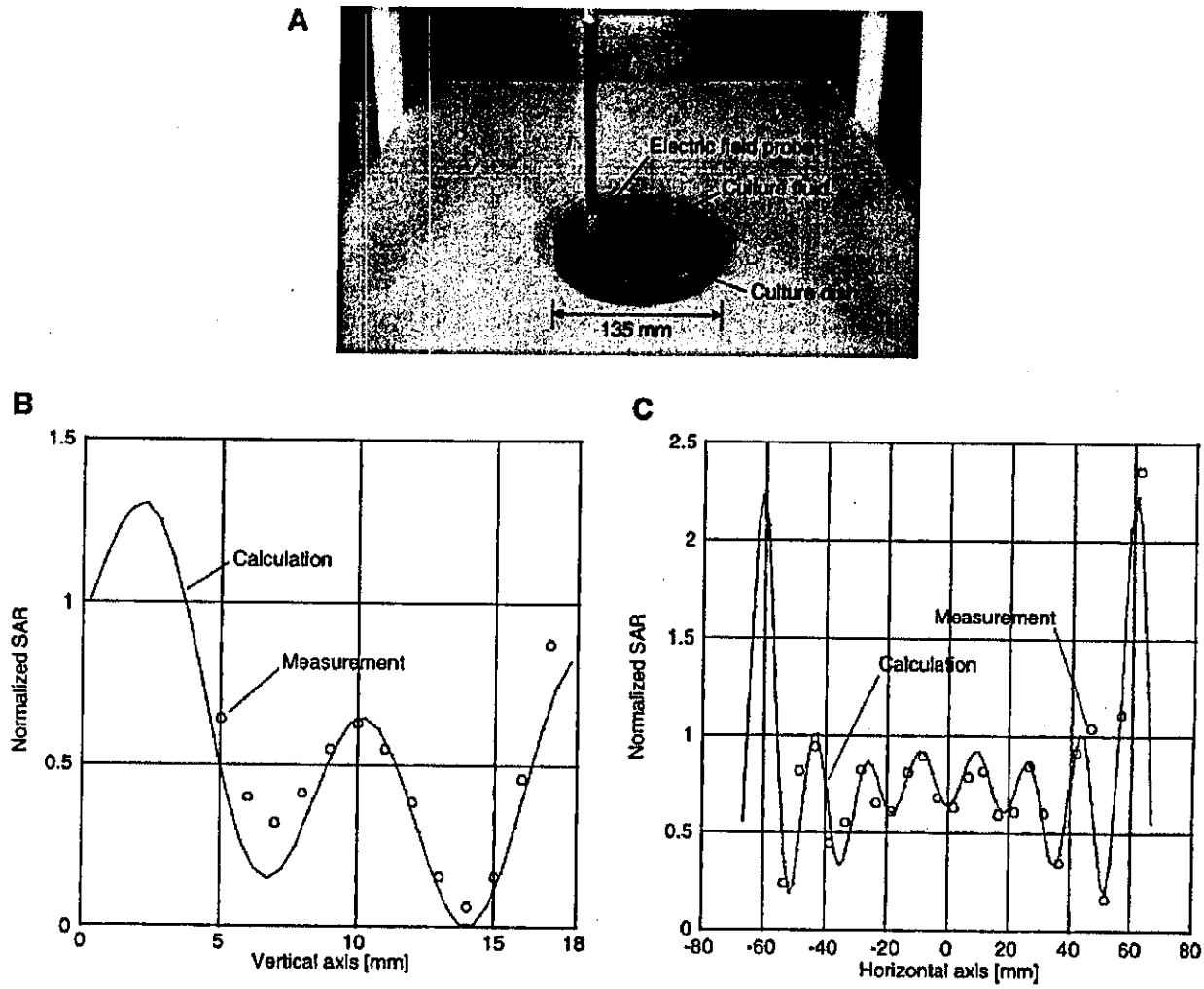

Fig. 2. Comparison of experimental and numerical SAR distributions in the culture fluid in a large (135 mm diameter) culture dish. **A**: Photograph of SAR measurement setup. **B**: The electric field probe scanned along the central vertical axis in 1 mm steps in the culture fluid. **C**: The electric field probe scanned along the horizontal axis 10 mm above the bottom of the dish, along a 5 mm grid in the culture fluid.

distance between the tip and the sensing elements of the electric field probe was approximately 5 mm. The normalized SAR distributions obtained by measurement and calculation along the horizontal axis at the height of 10 mm from the bottom of the dish are shown in Figure 2C. Moreover, two dimensional SAR distributions measured in the culture fluid at the height of 5, 10, and 15 mm from the bottom of the dish on a 5 mm grid revealed that the standard deviations are 19%, 25%, and 60%, respectively. The calculated values were respectively 19%, 31%, and 83%. Although the relatively low SAR level at the height of 15 mm from the bottom of the dish caused some difference in the standard deviation, the measured and calculated results were in good agreement. Totally, the effectiveness of the calculation was confirmed.

The SAR distribution in the culture fluid at the bottom of the 49 35 mm diameter Petri dishes is illustrated in Figure 3A. Moreover, Figure 3B shows the SAR distribution in the culture fluid for the cross section (illustrated as "X-X'" in Figure 3A) of the middle of the tray cutting through seven dishes. Figure 3C illustrates the histogram of the culture fluid at the bottom of the 49 35 mm diameter Petri dishes. Assuming that an incident electric field has the same amplitude as the average electric field in the exposure area for the antenna input

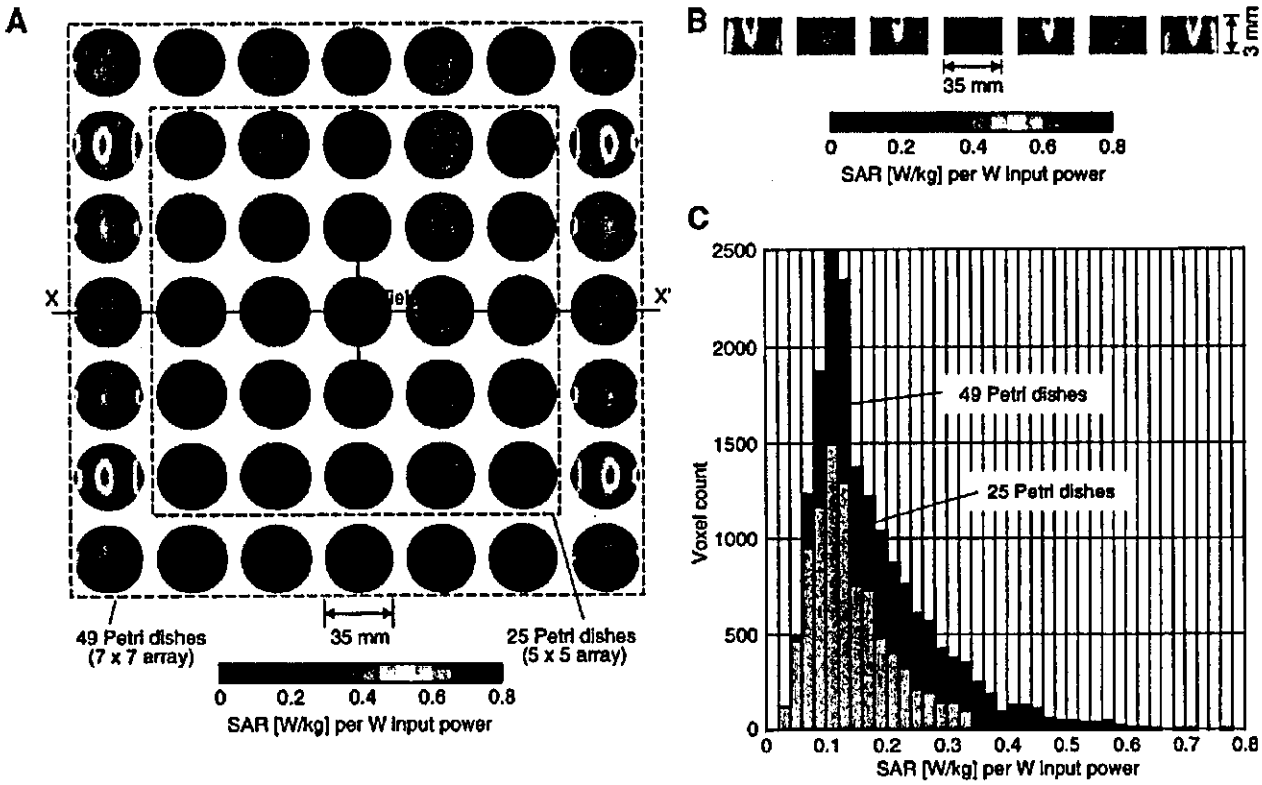

Fig. 3. Calculated SAR distributions in the culture fluid in Petri dishes used in the in vitro experiments (35 mm diameter). **A**: At the bottom of the 49 Petri dishes the electric field is depicted from the bottom up. **B**: A cross section (illustrated as "X-X" in Fig. 3A) of the middle of the tray cutting through seven Petri dishes. **C**: Histogram at the bottom of the 49 and 25 Petri dishes.

power of 1 W by measurement, the mean SAR was 0.175 W/kg. For example, to achieve a mean SAR of 0.08 W/kg, which corresponds to the basic restriction for general public exposure recommended by International Commission on Non-Ionizing Radiation Protection (ICNIRP) [ICNIRP, 1998], an antenna input power of 0.46 W is required. An antenna input power of 11.4 W leads to a mean SAR of 2.0 W/kg. The standard deviation of the SAR distribution was 59%. When the SAR distribution in the culture fluid at the bottom of the inner 25 Petri dishes (5 × 5 array) was evaluated as illustrated in Figure 3C, the mean SAR was 0.138 W/kg for an antenna input power of 1 W and the standard deviation of the SAR distribution was 47%. Here, the remaining 24 of the 49 Petri dishes were assumed to be exposed simultaneously.

Schönborn et al. [2001] summarized the standard deviations of the SAR distribution in the culture fluid at the bottom of 60 mm diameter Petri dishes in closed type RF exposure systems. TEM cells provide a standard deviation of approximately 15% at 835 MHz when only two dishes are used. At 1.62 GHz, a standard deviation of approximately 30% is achieved using an RTL and a rectangular waveguide. The standard deviations of the SAR distribution in open type RF exposure systems have not been previously reported in detail. However, Guy et al. [1999] reported that plane wave exposure resulted in a standard deviation of more than 100% at the bottom of four T-25 flasks at 2450 MHz. Our system provides an equivalent uniformity of SAR distribution compared to other systems. Large-scale cell culture corresponding to the placement of 49 35 mm diameter Petri dishes with reasonable SAR uniformity contributes to large scale in vitro experiments.

If the RF exposure level is relatively high, significant heat due to RF exposure in the culture fluid may be expected. Although air at 37 °C flows constantly in the culture room and excludes the heat in the culture fluid caused by RF exposure to a certain extent, we expect that we need to increase the capabilities of our system to exclude a significant rise in temperature due

to high level RF exposure in the culture fluid. When the mean SAR is 0.08 W/kg, we confirmed that the rise in the temperature in the culture fluid is less than 0.1 °C by the measurement using the fiberoptic thermometers and the calculation based on the heat conduction equation [Taflove and Brodwin, 1975b]. Therefore, for lower level RF exposure such as that by mobile radio base stations, there is no need to consider the temperature change in the culture fluid.

Cell Culture

The temperature change over 72 h in each culture room was less than 0.2 °C, whereas that in the machine room was more than 2.0 °C, as shown in Figure 4A. It was also confirmed that the temperature recovery in the culture room required 18 min to reach 36.5 °C and at most 72 min to reach 37.0 °C after opening the front door of the culture case for 30 s. Figure 4B shows the number of H4 cells in the incubators after 72 h. The cell count was 62 ± 8.1, 62 ± 4.6, and 60 ± 9.2 (mean $\pm$ standard deviation) $\times 10^4$/dish in RF exposure-incubators 1 and 2 (both sham exposed) and the common CO_2 incubator, respectively. No statistically significant difference was exhibited among them. A comparison of ATP levels after 72 h is illustrated in Figure 4C. The ATP levels were 8409 ± 2480, 8717 ± 1712, and 9065 ± 3021 nmol/dish in RF exposure-incubators 1 and 2, and the common CO_2 incubator, respectively. These results also showed no statistically significant difference among them. It was confirmed that the culture conditions of our RF exposure-incubators were equivalent to those of a common CO_2 incubator. Maintenance of the appropriate temperature, CO_2 density, and humidity realized stable long term culture conditions.

CONCLUSION

A beam formed RF exposure-incubator for in vitro experiments has been presented. Its important features are that the combination of an open type RF exposure source and a self-contained environmental control unit established large scale and long term cell culture conditions, and that a dielectric lens focused RF energy in the direction of the culture case and provided a uniform electric field as well as minimizing the size of the RF exposure system.

Since the electric fields are parallel to the culture fluid layer, the standard deviation of the SAR distribution in our system is slightly larger than those in systems where the electric fields are normal to the culture fluid layer. For example, our system achieves standard deviations of 59% and 47% for 49 and 25 culture dishes, respectively. The efficiency in our system, which is

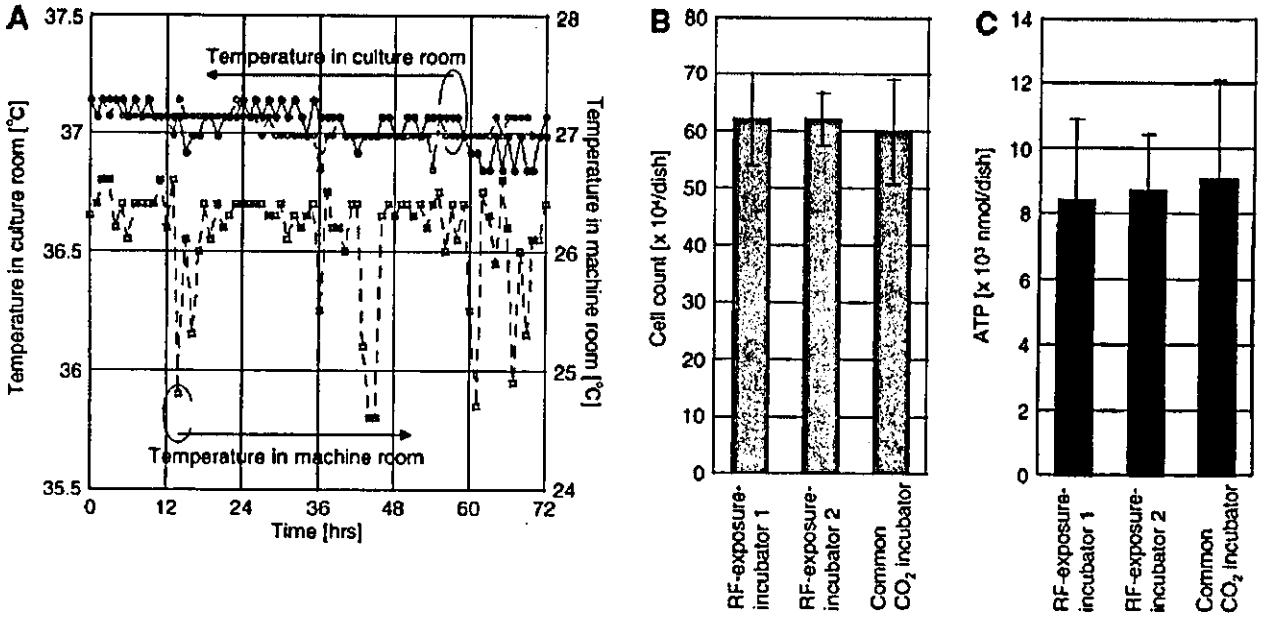

Fig. 4. Culture conditions in the RF exposure-incubators. **A**: Temperature regulation in two culture rooms over 72 h. **B**: Counts of the H4 cell lines in the two RF exposure-incubators and in the common CO_2 incubator after 72 h. **C**: ATP levels of the H4 cell line in the two RF exposure-incubators and in the common CO_2 incubator after 72 h.

equivalent to an RTL, provides a sufficient SAR level in our in vitro experiments with an acceptable standard deviation of the SAR distribution.

If the RF exposure level is at such a high level that it causes a significant rise in the temperature in the culture fluid, we expect that we need to increase the capabilities of our system to exclude a significant rise in temperature due to high level RF exposure. However, we have confirmed that a mean SAR of 0.08 W/kg at the bottom of the culture fluid, which corresponds to the basic restriction for general public exposure recommended by ICNIRP, causes a temperature rise of less than 0.1 °C. Therefore, our system can effectively simulate RF exposure from mobile radio base stations without the need to consider the heat in the culture fluid. In addition, to achieve a mean SAR of 0.08 W/kg at the bottom of the 49 or 25 culture dishes, input powers of 0.46 W or 0.59 W are required, respectively. This indicates that conventional amplifiers can be used to simulate such RF exposure of cells.

Large scale cell culture enables RF exposure of various cell lines and allows us to obtain highly accurate and reliable in vitro statistical data through the analysis of an extremely large number of cells. Observation of continual changes in the cells can also be realized. Using an RF exposure system, we will conduct in vitro experiments for low level, long term exposure by 2 GHz radiation, including gene analysis.

REFERENCES

Burkhardt M, Poković K, Gnos M, Schmid T, Kuster N. 1996. Numerical and experimental dosimetry of Petri dish exposure setups. Bioelectromagnetics 17:483–493.

Gajda GB, McNamee JP, Thansandote A, Boonpanyarak S, Lemay E, Bellier PV. 2002. Cylindrical waveguide applicator for in vitro exposure of cell culture samples to 1.9-GHz radiofrequency fields. Bioelectromagnetics 23:592–598.

Guy AW, Chou CK, McDougall JA. 1999. A quarter century of in vitro research: A new look at exposure methods. Bioelectromagnetics 20(Suppl 4):21–39.

Harrison GH, McCulloch D, Balcer-Kubiczek EK, Robinson JE. 1985. Far-field 2.45 GHz irradiation system for cellular monolayers in vitro. J Microw Power 20:145–151.

International Commission on Non-Ionizing Radiation Protection (ICNIRP). 1998. Guidelines for limiting exposure to time varying electric, magnetic and electromagnetic fields (up to 300 GHz). Health Phys 74:494–522.

Laval L, Leveque PH, Jecko B. 2000. A new in vitro exposure device for the mobile frequency of 900 MHz. Bioelectromagnetics 21:255–263.

Moros EG, Straube WL, Pickard WF. 1999. The radial transmission line as a broad-band shielded exposure system for microwave irradiation of large numbers of culture flasks. Bioelectromagnetics 20:65–80.

Schmid T, Egger O, Kuster N. 1996. Automated E-field scanning system for dosimetric assessments. IEEE Trans Microw Theory Tech MTT 44:105–113.

Schönborn F, Poković K, Wobus AM, Kuster N. 2000. Design, optimization, realization, and analysis of an in vitro system for the exposure of embryonic stem cells at 1.71 GHz. Bioelectromagnetics 21:372–384.

Schönborn F, Poković K, Burkhardt M, Kuster N. 2001. Basis for optimization of in vitro exposure apparatus for health hazard evaluations of mobile communications. Bioelectromagnetics 22:547–559.

Taflove A, Brodwin ME. 1975a. Numerical solution of steady-state electromagnetic scattering problems using the time-dependent Maxwell's equations. IEEE Trans Microw Theory Tech MTT 23:623–630.

Taflove A, Brodwin ME. 1975b. Computation of the electromagnetic fields and induced temperatures within a model of the microwave-irradiated human eye. IEEE Trans Microw Theory Tech MTT 23:888–896.

Taki M, Suzuki Y. 2002. Dosimetry for in vitro study on non-thermal biological effects of microwaves. Proc Int Symp Bioelectromagnetics 24–27.

The Role of DNA-Microarray in Translational Cancer Research

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Abstract:

The overall prognosis for the majority of cancer patients remains poor. Current conventional strategies in clinical cancer research are not able to adequately answer a large number of important questions. Although some patients achieve substantial benefits from classical cytotoxic chemotherapy others, will not. The mechanisms behind this phenomenon are still not in detail identified. Furthermore, the activity of promising novel molecular targeting anticancer agents like tyrosine kinase inhibitors is currently not predictable in the individual patient. The biological background for this clinical and prognostic heterogeneity in behaviour is more or less the large individual variation in the biological nature of tumors within the same classified histological subgroup.The usefulness of conventional histological classifications to predict patient prognosis or response to chemotherapy is definitely limited. The most promising way to improve this situation is to build clinical research strategies on intricate biological evidence. New genomic technologies have been developed within the recent years. These techniques are able to analyze thousands of genes and their expression profiles simultaneously. An increasing number of investigations has been reported looking at the application of these novel technologies within the setting of clinical trials. The purpose of this approach is to discover new subsets of cancer patients, to improve prediction of the clinical outcome of disease or response to treatment and select new targets for novel agents with innovative mechanisms of action based on the findings from gene expression profiles. Results of these gene expression profiling based studies could potentially lead to more individually tailored systemic cancer therapy. In the recent years a remarkable number of studies based on these techniques has already been reported. Although the published results are in general impressive and highly promising, a lot of work still remains to be done. Moreover, there is a strong need for an increase in reliability and reproducibility of gene expression profiling techniques and thus introduction of quality assurance in the performance of these assays. Although a large number of issues remain to be clarified prior to a more general application of genomic profiling techniques in clinical cancer research, this strategy will eventually turn out as a promising approach to improve successful management of cancer patients.

Introduction:

The overall prognosis for the majority of cancer patients is still rather unsatisfactory.Hardly any stage IV lung cancer patient is alive five years following following initial diagnosis [Mountain 1997].Even new generation cytotoxic agents with higher efficacy and more favourable toxicity profiles such as paclitaxel, docetaxel and gemcitabine have not brought an identifiable breakthrough in cancer therapy [Schiller 2002]. A large group of tumor entities is primarily resistant or will develop secondary resistance to cytotoxic chemotherapy. On the other hand, there is a definite subset of patients with an identifiable benefit from cancer chemotherapy. The basic mechanisms behind this clinical phenomenon remain not clearly identified. Adjuvant chemotherapy following definitive local treatment (e.g complete resection) of early disease is another important issue. In earlier stages with the opportunity for intended curative surgical treatment, there is currently no reliable method to predict which patient will have a significant benefit from adjuvant treatment.The current situation regarding the use of novel molecular targeting drugs is of striking parallelity. Activity of these agents is at the moment not at all be predictable in individual patient. A substantial reason for this situation in current clinical cancer research are the remarkable individual varities in the biological nature and clinical behaviour of tumors even within the same pathological entity. The usefulness of classical histological subclassifications to predict patient prognosis or response to chemotherapy is definitely limited. Introducing more information on tumor biology from basic research results to clinical investigations is supposed to improve cancer treatment strategies in the future. This approach could be one important step to individualize cancer management. New genomic technologies have been developed within the recent years. These techniques have the capability to analyze the expression and activity of thousands of different genes simultaneously. An increasing number of investigations has applied these genomic techniques as an adjunct to their clinical studies with the

purpose to discover new (sub)-classes of tumors or predict outcome of therapy on the basis of these gene expression profiles. Although a number of studies has been published during the last years with impressive and clinical relevant results, a lot of work remains to be done. One major challenge will be to find the appropriate statistical method for analyzing the large data sets correctly to get valid and reliable scientific results. Currently, another major problem is the lack of comparability between results from different investigations. Several different genomic techniques (cDNA-microarray, filter-array, short and long oligonucleotide arrays), statistical methods (supervised and unsupervised analysis) have been used in recent clinical investigations. International standardizations of gene profiling based studies is needed for a proper interpretation of results in the future. In spite of a large number of unsolved issues when applying genomic techniques to clinical cancer research, these methods still belong to the most promising tools to improve treatment results within the future. The current role of genomic techniques in translational cancer research will be analyzed in the following overview. In the first part of this paper we will summarize the most important studies for frequent cancer entities published. Furthermore, the applied methods and their clinical relevance will be critical reviewed. In the second part of the manuscript, we will focus on the reliability and the reproducibility of reported genomic array data.

Breast Cancer

In 2000 a Norwegian – American Cooperative Group performed an analysis of breast cancer cell lines and tumor tissue based on DNA microarrays [Perou 2000]. They could classify the tumors into subtypes distinguished by their different gene expression profiles using the "hierarchical clustering [1]" methology based on 1753 genes independent from the histological classification The tumors were subdivided into an ER+(estrogen-receptor-positive)/luminal like, a basal-epithelium-

like group, an ERB-B2-(erythroblastic leukemia viral oncogene homolog 2) group and a normal-breast-like group. The same group published within the following year a further investigation on this subject [Sorlie 2001]. This time, in a larger number of tumors at least six subtypes could be differentiated on the basis of their gene expression patterns using an intrinsic set of 457 genes (Table 1).The previous (ER+)/luminal-like group could be subdivided into three prognostic groups. In addition, a list of 256 genes related to clinical outcome was developed using a special supervised data-analysis (SAM: significance analysis of microarray, this technique will be explained more detailed in the second part of this article) derived from a clinical data set of 76 carcinomas, where sufficient data were available. This 256-gene-"predictor" was correlated with clinical outcome in a subcohort of 49 patients with locally-advanced breast cancer uniformly treated in a prospective clinical study. The basal-like subtype was associated with a poorer prognosis regarding overall as well as relapse-free survival (Table 1). Interestingly, significant differences could be observed in the clinical outcome between two different estrogen–receptor-positive groups. West and co-workers could demonstrate the potential usefulness of DNA microarray analysis to discriminate different breast cancer patients on the basis of their ER (estrogen-receptor) status (Table 2)[West 2001]. A 100-gene "predictor" to estimate estrogen receptor status was developed analysing a set of 38 samples (the "trainings set"). Then in a second step, the "predictor" was validated in a set of nine independent tissue samples (the "test set") (Table 3). In some samples, the "predictor" could adequately predict clinical ER-status. Five samples with conflicting results of immunohistochemistry and immunoblotting regarding the ER-status were included within this "test set". In some of these samples, the predictive probability of the "predictor" was found lower. This could be reflect a heterogeneity of expression within the individual tumor. Furthermore, impressive data regarding an analysis of estrogen-receptor status by calculating gene expression profiles were published by a Swedish group within the same year

(Table 2) [Gruvberger 2001]. A 100-gene "predictor" for ER status was developed in a "trainings set" of 47 tumors based on microarray data using "artificial neural network[2]"(Table 3). The "predictor" was validated in 11 independent samples of a "test set". All 11 tumors could be classified correctly by this 100-gene marker. Interestingly, even without the top discriminator genes, including ER itself, the "artificial neural network" could adequately predict ER-status.

Adjuvant cancer therapy is well established in the treatment of breast cancer. Chemotherapy or hormonal therapy is able to reduce the risk of disease dissemination in one-thirds of the patients, but a large number of patients would have already been cured without the application of adjuvant therapy. Taking into account that application of adjuvant chemotherapy carries a well-defined morbidity and mortality risk, a proper selection of patients with an established benefit from adjuvant chemotherapy would be extremely helpful. Based on the hypothesis, that patients with a poor prognosis following surgery would get benefit most from adjuvant therapy a Dutch group performed a translational study testing the predictive impact of DNA Microarray data on overall survival prognosis in young female with primary-lymph–node-negative breast cancer [van 't Veer 2002]. In this study, tumor tissue from 117 young patients with primary lymph-node-negative breast cancer was analysed by DNA microarray technique. A 70-gene prognosis "predictor" ("poor prognosis signature") for patients with a short interval to development distant metastasis was established by supervised classification of the gene expression profiling. This "poor prognosis signature" included genes regulating cell cycle, invasion, metastasis and angiogenesis. A second correlation study was undertaken by the same group to confirm the predictive power of this 70-gene prognostic marker in an larger and less homogeneous group of patients [van de Vijver 2002]. A series of 295 consecutive patients with stage I and II breast cancer, who underwent surgery, were included into this study. All patients included were younger than 53 years. In this investigation patients with-lymph-node-negative (151 pts) and lymph-node-positive

(144 pts) disease were analyzed. A group of 180 patients with "poor prognosis signature" could be separated from 115 patients with "good-prognosis-signature" (Table 4). The overall 10-year survival rate was found to be 54.6 percent within the "poor" and 94.5 percent in the "good prognosis group". The probability to remain free from distant metastases within 10 years was found to be 50.6 percent in the group with "poor-prognosis- signature" and 85.2 percent in the group with "good-prognosis-signature". In comparison to the "good prognosis group" the estimated hazard ratio for developing distant metastasis in the group with "poor-prognosis-signature" was 5.1(95 percent confidence interval, 2.9 to 9.0; $P< 0.001$). This microarray based prognosis profile was identified as a strong independent factor in predicting disease outcome. Interestingly, the prognostic profile did not depend on lymph–node status, well-known to be one of the classical prognostic factors. On the other hand, the positive-estrogen–receptor status, the other classical predictor, was strongly correlated with a "good prognosis signature" profile. In spite of these impressive results, there has been important criticism towards these findings: An insufficient analysis of intratumoral representativeness of the tumor samples and the measurement of tumor size is critically discussed [Kunkler 2003, Kopans 2003]. However, this pioneer investigation has proven, that -in principle- a prediction of clinical outcome based on microarray data may be possible.

Lung Cancer

Lung Cancer is still the leading cause of cancer-related deaths in the industrialized world [Jemal 2002]. Physicians treating patients with this disease often face difficult decisions to be made within all clinical disease stages. For example, the issue of adjuvant chemotherapy following complete resection is not conclusively solved with conflicting results published [Scagliotti 2003; International Adjuvant Lung Cancer Trial Cooperative Group 2004]. A reliable method to predict

patient prognosis following surgical treatment of early stage lung cancer could be most helpful to estimate the benefit of adjuvant chemotherapy for an individual patient. Classical histopathology is definitely insufficient for this decision making. Beer published in 2002 a translational research study correlating gene expression profile data with overall survival in patients with early stages lung adenocarcinoma after surgery [Beer 2002]. Tumor tissue of 86 primary adenocarcimas of the lung, including 67 stage I and 19 stage III tumors, was investigated. As an internal control 10 non-neoplastic lung tissue samples were analyzed. Using "hierarchical clustering" methology, three different patient groups with association of cluster and stage (P=0,030) or tumor differentiation (P=0,01) could be differentiated. All ten non-neoplastic tissue samples could be clustered within the same patient group. In addition, the authors could derive a 50-gene-risk-index by identifying survival related genes using univariate Cox analysis (Tab.5).When calculating the 50-gene-risk-index and grouping the patients based on the results, significant differences in overall survival between the individual groups could be identified. Grouped "high-risk" and "low-risk" stage I adenocarcinomas differed significantly between each other (p=0.003), whereas low- and high risk stage III tumors did not. The robustness of the 50-gene-risk-index in predicting overall survival in early stage lung adenocarcinoma was tested in an independent data set of 84 tumor samples and related to the survival. A high and a low risk group could be separated (P=0.003). Interestingly, among the 62 stage I tumors in this analyzed population, high- and low-risk groups could be observed differing significantly (P=0.006) in their overall survival duration. In conclusion, the authors postulate, that the identification of a high risk group within stage I lung cancer patients would lead to the consideration of a postoperative adjuvant intervention for this group. In 2003 a Japanese group has published another important cDNA microarray based study regarding lung cancer [Kikuchi 2003]. A set of 37 tumor tissue samples of non-small cell lung cancer patients were analyzed. To avoid investigation on non relevant

tissue they only analyzed cancer cells selected by laser capture microdissection .The most frequent NSCLC-subtypes adenocarcinoma and squamous cell carcinoma could be easily distinguished by applying a clustering algorithm to the expression data results. To explore gene expression in post-chemotherapeutic lung cancer tissue, a small pilot-study using RNA filter-array was performed in our institution [Ohira 2002]. Lung and normal tissue from three patients who underwent neoadjuvant therapy prior to surgery were collected following the end chemotherapy. Gene expression data obtained by a 588 gene filter arrays were analysed by "hierarchical clustering" method. Remarkably, normal tissue and tumor tissue from the same patient showed more similarities and clustered nearer than normal and normal or tumor and tumor samples from different patients "Fig. (1)".On the other hand, groups of genes significantly differed in expression profiles between normal and malignant tissue. Especially angiogenesis and invasion related genes were up-regulated in the tumor samples "Fig (2)" These results suggest that molecules involved in angiogenesis are suitable targets for novel drugs administered following chemotherapy. This early study is one example for how genomic techniques could help to discover new candidates for target based therapeutics in the future.

Gastrointestinal Cancer

Gastric cancer is still the fourth leading cause of cancer in the world [Parkin 2001]. Due to the lack of sufficient systemic control induced by current anti-neoplastic agents surgery remains the cornerstone treatment approach in this disease. The development of lymph node metastasis is a well established independent risk factor for recurrence of gastric cancer. In the recent years, two independent research groups have established microarray based risk factor scoring systems for the development of lymph-node metastasis in gastric cancer. In 2002, a Japanese group published

a study in patients with Intestinal Type Gastric Cancer [Hasegawa 2002]. Primary gastric cancer and corresponding noncancerous gastric mucosa from 20 patients who underwent surgery were comparatively analyzed. A set of 61 genes that were commonly up-regulated and 63 genes down-regulated in Intestal-Type Gastric Cancer in more than 75% of the cases could be identified. In a second step the expression profiles of nine cases with and cases without lymph-node metastasis were compared. In this approach, 12 genes that were differentially expressed (P< 0,01) were identified by employing a random permutation test. Nine of these 12 genes were over-expressed and three were down-regulated in node positive tumors. By use of a "stepwise discriminant analysis" five independent "predictors" were identified among these 12 genes (Table 6). The predictive scoring system was confirmed in nine independent additional tumor tissue samples. All nine cases (four node positive and nine node negative) were correctly assigned to each class by the means of the scoring system.

One year later, a Dutch group performed a comparable investigation. The molecular data of 35 gastric carcimomas were analysed with their clinical data sets [Weiss 2003]. Microarray Comparative Genomic Hybridization (GCH), which allow to analyze accumulation of genetic changes that to a large extend occur on a chromosomal level was applied to their approach. Three different groups could be distinguished by "hierarchical clustering" of the microarray CGH results. For each cluster they could define a signature of 204 genes by using a "leave one out" cross validation. Each cluster was analyzed for correlation with clinico-pathological data. The lymph–node status and the overall survival were criteria with significant differences between the individual groups. In one group significantly less lymph-node positive cases (40%) were found than in the other one (83% in comparison to 88%). Patients who belong to the former group were found to have a significantly longer survival duration (P=0,019).

Both cited studies focussed on a clinically most important issue. The discussion about the inclusion of extended lymph node disssection into surgery for gastric cancer remains rather controversial [Bonenkamp 1999,Cuschieri 1999]. The increase of overall morbidity and mortality associated with this treatment strategy has to be well balanced against the benefit for lymph-node positive patients following this intervention. Predicting the overall risk of lymph-node metastasis by microarray techniques in an individual patient could be a reasonable strategy to select patients for this kind of therapy in the future and would have major implications for clinical practice in this entity.

In 2003 Suganuma published a study focussed on possible chemoresistance-related genes in gastric cancer [Suganuma 2003]. Tumor samples and corresponding normal mucosa from 35 patients with advanced gastric cancer were differentially examined. The in vitro sensitivity of cells from each dissociated tumor sample against cisplatin, 5-flourouracil, mitomycin C and doxorubicin was measured by MTT (4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay. The results obtained from in-vitro cytotoxicity testing assays were correlated with the results of cDNA microarray analysis of corresponding tissue samples. In the case of cisplatin, "hierarchical clustering" could successfully distinguish "sensitive" and "resistant" tumors from each other. A set of 23 potential "cisplatin-resistance-related-genes" could be selected by this method. The latter group included vascular permeability factor, two membrane-transporting subunits and retinoblastoma-binding protein. In a further selection based on strong criteria, metallothionein IG and heparin-binding-epidermal-growth-factor-like-growth factor were also identified as candidates for cisplatin-resistance-related genes. Within this approach dihydropyrimidine dehydrogenase and HB-EGF-like growth factor were suggested to be 5-FU resistance-related genes. In this innovative approach the authors could demonstrate that DNA-microarray could be useful to investigate drug-resistance and would be a means to understand

some of the complex mechanisms behind it. Although this study is only based on a limited data set this method could in principal be an important step for an individualized and customized cancer therapy, in the future.

Moreover, in 2003 a Japanese group published a correlative study based on gene expression profiling in patients with colorectal cancer [Tsunoda 2003]. To clarify the regulatory factors in this malignant disease, differential gene expression profiles were analyzed by filter-array in surgically resected specimen (tumor and normal mucosa) obtained from ten patients with colorectal cancer. The correlation between several clinico-pathologic factors and cancer-related genes were investigated by using complex statistical analyses including "average linkage hierarchical clustering and principal component analysis (PCA)[4]". As an example, the c-myc- binding- protein and the c-jun-proto-oncogene were both identified as possible correlative markers for histological differentiation and overall clinical prognosis "Fig.(3)".The early-growth-response-protein 1 was selected to play an important role in the progression of clinical stage. The authors concluded, that PCA was identified as an appropriate method to select candidate genes relevant to predict clinico-pathologic factors in a small population of clinical samples from colon cancer patients.

Lymphoma:

Diffuse large B-cell lymphoma (DLBCL) presents the most common subtype among the lymphoid neoplasm in adults (International Lymphoma Study Group 1997). Less than 50% of the patients are currently cured with standard combination chemotherapy [Popat 1998]. In 2000 the first large gene expression profiling study was performed in this disease .[Alizedah 2000]. Within this study, different types of diffuse large B-cell lymphoma patients could be identified by gene expression profiles. A specific microarray assay for lymphoma was designed. This "Lymphoma-

chip" included genes preferentially expressed in lymphoid cells and genes with known or suspected roles in cancer development and immunology. Using this array 96 normal and different malignant lymphocyte samples were comparatively analyzed. All DLBCL samples could clearly be separated from normal lymphocyte samples and other lymphomas. Focused on genes related to a separate stage of B-Cell differentiation and activation, a germinal-centre-B-like DLBCL and an in vitro-activated-B-like DLBCL could distinguished among the group with diffuse large B-cell lymphoma by "hierarchical clustering" analysis. This clustering was based on the hypothesis that DLBCL derives from normal B-cells within the germinal centres (GC). As a consequence to this, overall survival and clustering to one of these two groups were correlated. Patients belonging to the GC-B-like-group had a significantly higher five-year survival rate than patients belonging to the activated-B-cell-DLBCL group ($P< 0,01$). The average five-year survival rate for all patients was 52%, for patients of the GC B-like DLBCL 76% and for patients of the activated B-like DLBCL only found to be 16%. Two years later, Shipp and co-workers published another prognostically predictive score for DLBCL based on gene expression profiling [Shipp 2002]. Contrary to the latter work an alternative strategy that was independent of an *a priori* hypothesis was employed. A novel "supervised learning method" was applied. Tumor samples from 77 patients were analysed. 58 patients with DLBCL and 19 patients with Follicular Lymphoma were clearly distinguishable by use of this method. Clinical outcome prediction in DLBCL patients based on gene expression profiling data was the further purpose of this study. The long-term follow up was available for all DLBCL patients. While 32 patients were eventually cured, 26 patients turned out to have fatal or refractory lymphoma disease. An "outcome predictor" was designed using a "supervised learning classification approach" (weighted voting algorithm and cross validation test). The highest accuracy was obtained using a "predictor" set of 13 genes (Table 7). Using this "predictor" set the DLBCL collective could be