Identification of Biomarkers for Radiation Response Using cDNA Microarray

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Abstract

DNA damage by physical insult including UV and g-radiation might provoke genetic alterations in cells, which is followed by either acute cell death or tumorigenesis. The responsiveness to g-radiation depends on cellular context of target cells. To understand the mechanisms of checkpoint control, repair and cell death following genotoxic stimuli, cDNA microarray can provide the gene expression profile. To make a profile of gene expression in irradiated Jurkat T cells, we hybridized the cDNA microarray using cDNA from g-irradiated Jurkat T cells. Jurkat T cells were exposed to 4Gy to 16Gy, and total RNA were extracted at 4 to 24 hrs after irradiation. The hybridization of the microarray to fluorescence-labeled cDNA from treated and untreated cells was analyzed by bioinformatic analysis to address relative changes in expression levels of the genes present in the array. Responses varied widely in different time points, suggesting acute stress response and chronic restoration or cell death. From these results we could select 384 genes related to radiation response in T cells, and radiation response might be different in various types of cells. Using RadChip, we could separate "the exposed" from control PBMCs. We propose that RadChip might be useful to check the radiation research as well as radiation carcinogenesis.

CV

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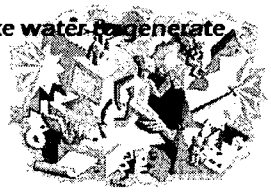
Mechanism of ionizing radiation impacts on cells

➢ *Directly impacts target molecules or transfer energy*

e.g. DNA strand breaks

➢ *Indirectly impacts others like water to generate free radicals*



Doses and limits

➢ **rad**, *radiation absorbed dose*

100 rad = 1 Gray or 100cGy = 1J/Kg

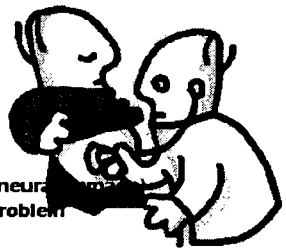
Chest X-ray = 0.1 rad

Acute sickness at 50-100 rad at once

➢ **rem**, *measure of relative danger*

Average exposure : 0.2 ~ 0.4 rem/yr

Health Effect



Acute (hours to weeks)

- visual flashes (maybe due to neura
- headache, malaise, sensory problem
- nausea, vomiting, diarrhea
- damage to mitotic tissues
- even to death

Delayed (months to years)

- cataract
- general tissue damage and mutations
- cancer induction
- impaired fertility
- heritable effects
- developmental abnormalities

Issues on health effects in radiation biohazard

- *genetic susceptibility in individual or subpopulation*
- *early and sensitive detection of exposed group*

Suitable biomarkers for radiation response

SELECTION OF RADIATION-SPECIFIC RESPONSES

AMUNDSON SA, DO KT, SHAHAB S, BITTNER M, MELTZER P, TRENT J, FORNACE AJ JR. (2000) IDENTIFICATION OF POTENTIAL MRNA BIOMARKERS IN PERIPHERAL BLOOD LYMPHOCYTES FOR HUMAN EXPOSURE TO IONIZING RADIATION. RADIAT RES. 154(3):342-6.

MATERIALS & METHODS

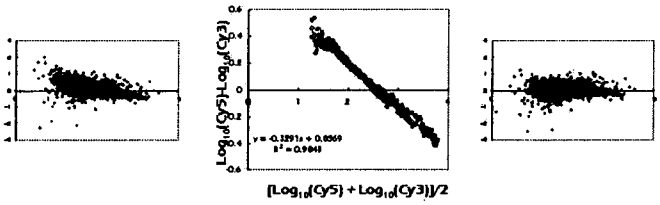
- **Cells**
  1. *Jurkat human T cell lymphoma*
  2. *Human peripheral blood mononuclear cell*

- **γ-irradiation**
  5X10^5/ml at 16 hours prior to γ-irradiation
  1-16 Gy using Cesium irradiator at SNUMC

- **cDNA microarray**
  - 40μg total RNA using Trizol
  - preparing first strand cDNA by AMV reverse transcriptase with a $(dT)_{24}$ primer and Cy3- or Cy5-labeled dUTPs.
  - 9,216 human cDNAs in a slide (4608 genes in duplicate) manufactured by Macrogen Inc.

DATA ANALYSIS

Fluorescence intensity was processed and measured by ImaGene v4.0 software (BioDiscovery Ltd., Swansea, UK), and the data were imported into an Excel (Microsoft) database with corresponding names for analysis and normalized by "Rank Invariant Method".. The clustering and display programs (http://rana.stanford.edu/software) developed by Eisen et al. were also used for analysis. Data was also processed by GeneCluster v1.1 (Whitehead/MIT Center for Genome Research).



RANK INVARIANT METHOD

Issues in cDNA microarray analysis : quality filtering, channel normalization, models of variations and assessment of gene effect

Tseng et al.(2001) Nucleic Acids Research

When the number of genes is small,

$S=\{ g: | \text{rank}(Cy5) - \text{rank}(Cy3) | < d \ \& \ \ell < \text{rank}[(Cy3+Cy5)/2] < G-\ell \}$

When the number of genes is large,

First selection

$S0 = \{g | \text{rank}(Cy5)-\text{rank}(Cy3) | < pxG \ \& \ \ell < \text{rank}[(Cy3+Cy5)/2] < G-\ell \}$

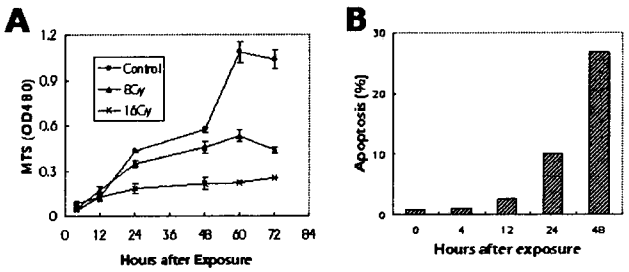
Then at each iteration we select

$Si = \{g: g \in S_{i-1} \ \& \ | \ \text{rank}_{g \in Si-1}(Cy5) - \text{rank}_{g \in Si-1}(Cy3) \ | < p \times |S_{i-1}| \}$

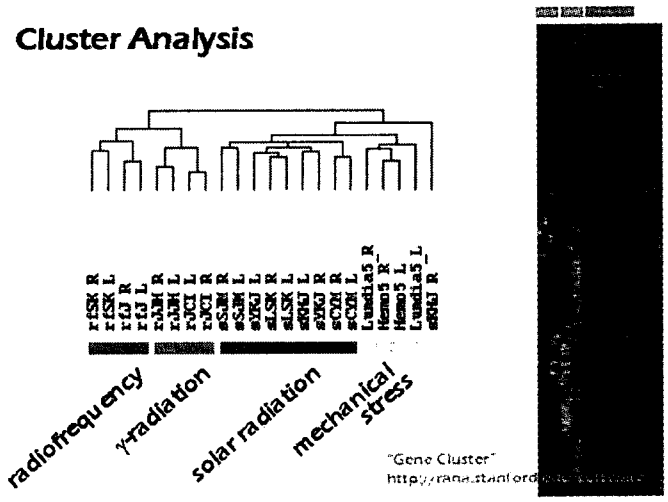
$|Si|$ is the number of genes in set Si

The iteration stops at the k th step when $|S_k| = |S_{k-1}|$ and The set of genes S_k is the chosen rank invariant set.

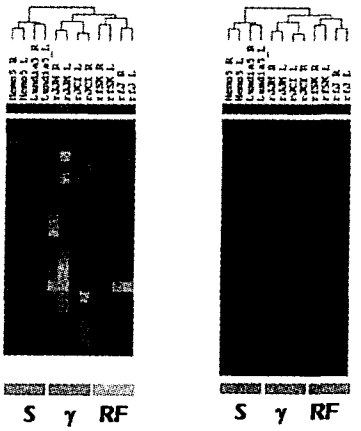
Cell proliferation and apoptosis of Jurkat T cells after ionizing radiation



Cluster Analysis

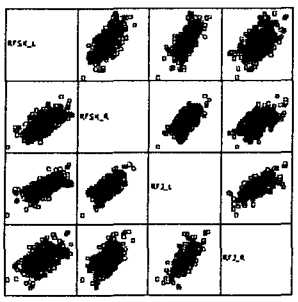


Cluster Analysis

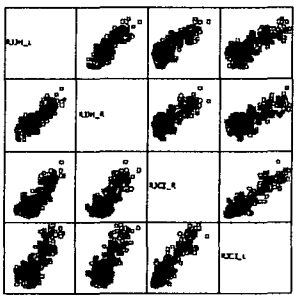


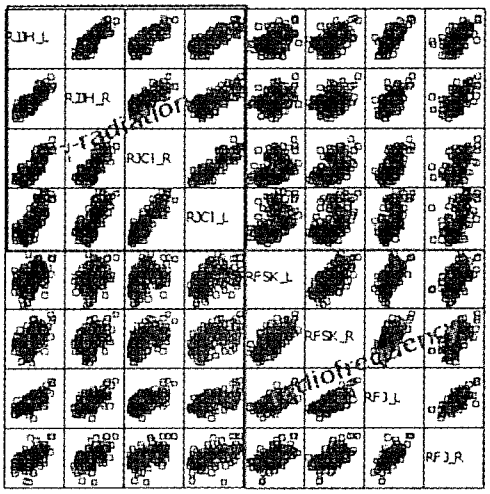
"Gene Cluster" http://rana.stanford.edu/software

RF vs RF

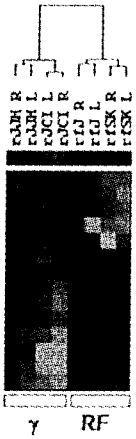


γ-radiation vs γ-radiation





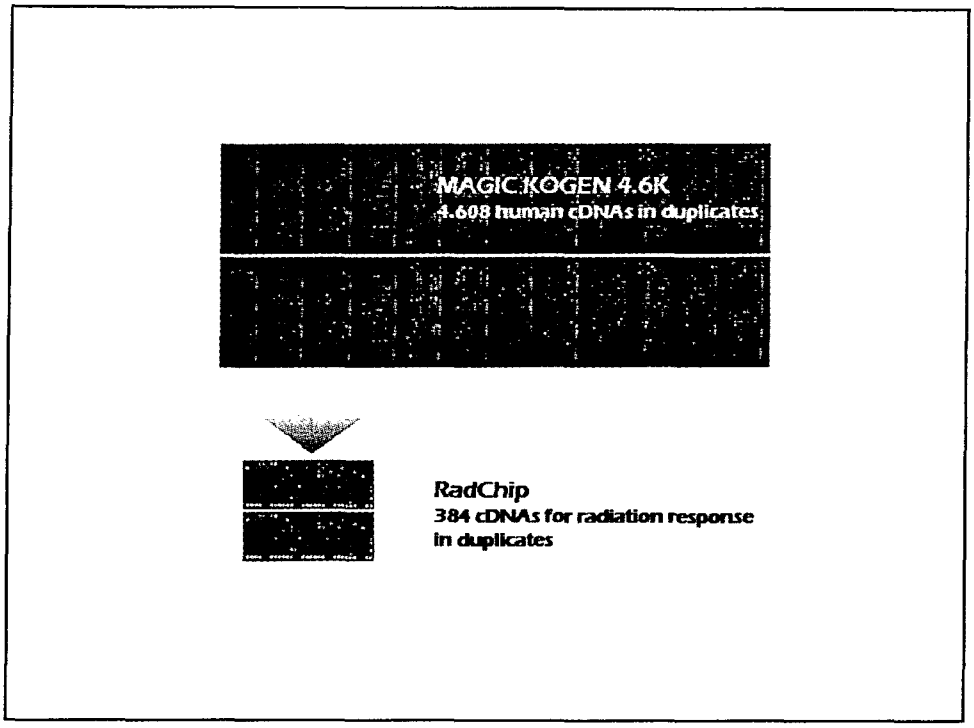
Cluster Analysis



For RF
Jurkat T cells
1.7 GHz, CDMA
75W/Kg SAR for 1 hour
After 4 hours incubation

For γ-radiation
Jurkat T cells
2Gy γ-irradiation by Cesium Irradiator,
After 4 hours incubation

"Gene Cluster"
http://rana.stanford.edu/software



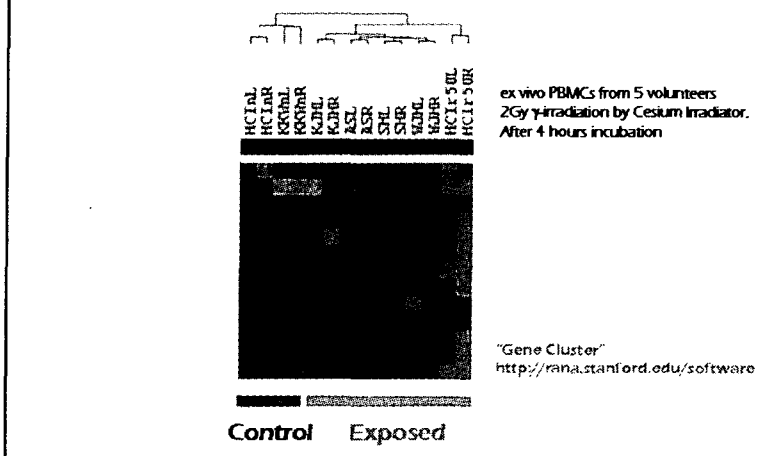


Figure 3. Genes distinguishing "the exposed" from control

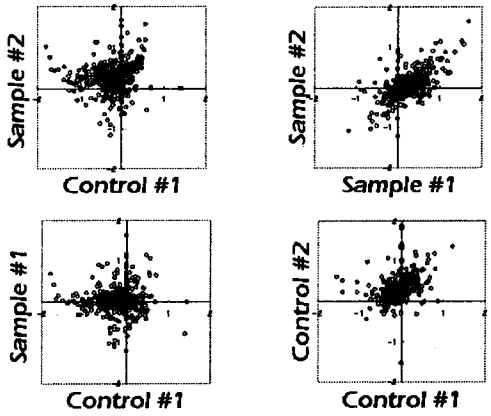


Figure 4. Scatter plot of 384 genes from sample and control

0-4-12-24-48 hours

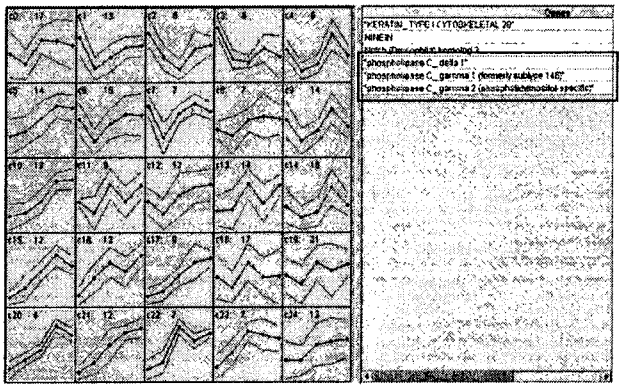
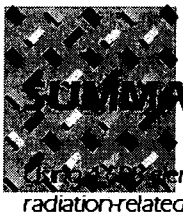


Figure 4. Self-Organizing Map analysis of time-dependent gene expression in Jurkat T cells at 2 Gy



SUMMARY (1)

Using cDNA microarray human cDNA microarray, we screened the radiation-related genes in Jurkat T cells, and selected radiation-specific gene expression.

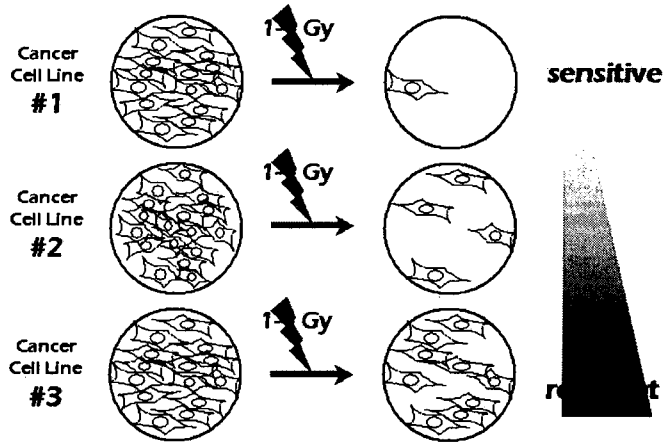
We fabricated RadChip containing 384 genes related to radiation response.

Using RadChip we could separate ex vivo irradiated human PBMCs from unexposed samples.

DIFFERENTIAL RESPONSES TO RADIOTHERAPY

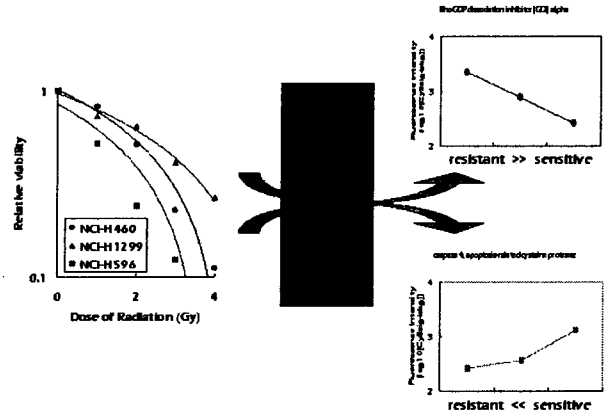
- HALF OF CANCER PATIENTS WERE SUBJECTED TO RADIOTHERAPY COMBINED WITH SURGICAL RESECTION IN THE UNITED STATES.
- UNRESPONSIVENESS MIGHT OVERRIDE THE BENEFICIAL EFFECT OF RADIOTHERAPY IN SOME PATIENTS.
- WE NOW GATHERED THE INFORMATION ON RADIATION RESPONSE LIKE THE INVOLVEMENTS OF P53, P21, G PROTEINS, INSULIN-LIKE GROWTH FATORS, ACID SPHINGOMYELINASE AND SO ON.
- USING CDNA MICROARRAY, WE TRIED TO MAP THE RADIO-RESISTANCY IN TERMS OF MOLECULAR CONTEXT OF EACH TUMOR TYPES.

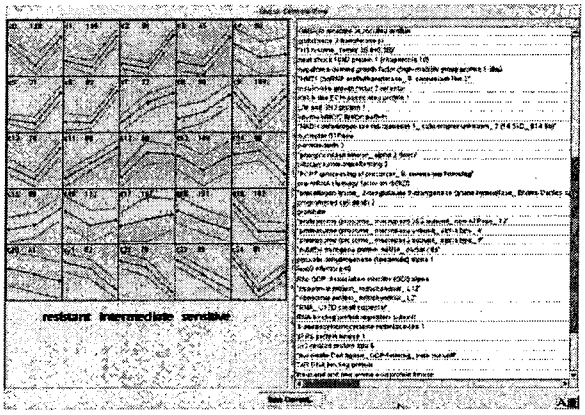
Colony Forming Assay; method



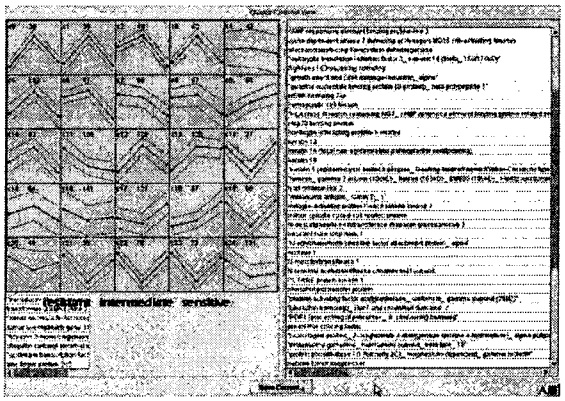
cDNA microarray ; methods

1. DNA chip :
   - Containing 4.6K human cDNA (Macrogen Inc.) in duplicate
2. Sample :
   - Total RNA from 9 different cell lines
   - Direct labeling during RT
   - Cy3-labeled samples without reference
3. Analysis :
   - Filtering and normalization
   - SOM

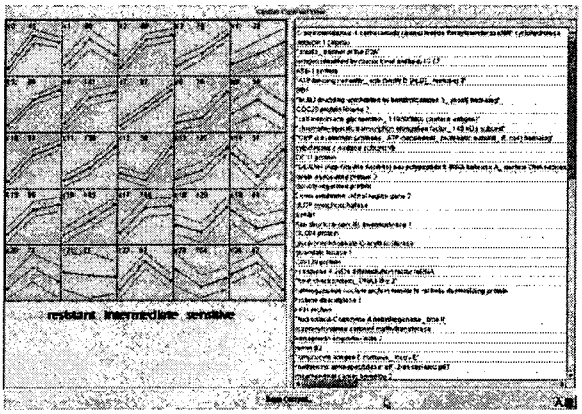

Relative viability
1
0.1
0
2
4
Dose of Radiation (Gy)
NCI-H460
NCI-H1299
NCI-HS96
resistant >> sensitive
resistant << sensitive


resistant intermediate sensitive

"Self-Organizing Map" analysis of gene expression profile of cervix cancer cell lines : HeLa - HeLa229 - CaSki



"Self-Organizing Map" analysis of gene expression profile of breast cancer cell lines : HS578T - SK-BR-3 - MDA-MB-231



SUMMARY (2)

...ns of gene expression in radio-resistant cell lines of ... seems to be different.

Tumor-specific lists related to radiation responses were tabulated and used for fabrication of customized DNA chip.

Various G proteins were commonly increased in radio-resistant cell lines, and radio-sensitive cells showed increased level of TNF-related genes or caspases.

We selected 200~400 genes for each type of tumors to make a DNA chip and need to qualify the predictability using in vivo samples like surgical specimens for radiotherapy.

Acknowledgement

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