Predicting the tumor response to radiotherapy using microarray analysis (Review)

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Abstract. Predicting the tumor response to radiotherapy is one of the major goals of human cancer treatment. Identification of the genes that are differentially expressed between radio-sensitive and radioresistant cancers by global gene analysis may provide new insights into the mechanisms underlying clinical radioresistance and improve the efficacy of radiotherapy. In this study, we reviewed the published reports identifying sets of discriminating genes using microarray analysis that can be used for characterization and the prediction of response to radiotherapy in human cancers. These reports indicate that many of the identified genes were associated with DNA-repair, apoptosis, growth factor, signal transduction, cell cycle and cell adhesion. Several genes were found to be predictors of the radiation response with various cancers and certain sets of identified genes were also found to predict the radiation response by using clustering analysis. Global gene expression profiling of responders and non-responders can be useful in predicting responses to radiotherapy and may also provide insights into the development of individualized therapies and novel therapeutic targets.

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1. Introduction

Radiotherapy is one of the major treatments for patients with various cancers. The general rationale for radiotherapy is based on the findings that radiation can inhibit cell proliferation or induce apoptotic cell death *in vitro* and inhibit tumor growth *in vivo* (1). However, the tumor response to radiotherapy sometimes differs among individual tumors even with the same histological field, and the lack of knowledge regarding the determinants of response has handicapped the development of predictive assays and the development of novel treatment strategies. Therefore, identification of the determinants of the tumor response to radiation has long been a goal of radiation oncologists and biologists.

Recently, an association between radioresistance and the expression of several genes was observed, with candidate genes, such as *p53* (2), *ras* (3), *raf-1* (4), *bcl-2* (5) and *survivin* (6). Furthermore, cancer stem cells have emerged as a contributor to radioresistance through the preferential activation of the DNA damage checkpoint response and an increase in DNA repair capacity (7). Although such discoveries have helped develop a partial understanding of the molecular mechanisms responsible for cellular radiosensitivity, the entire process remains to be elucidated. The advent of microarray gene expression technology permits simultaneous analysis of the expression levels of thousands of genes (8-10). Therefore, a study on molecular genetic events related to radiosensitivity can be conducted. This research can lead to the identification of gene regulatory pathways that result in the development of resistance to therapeutic procedures. To date, differential gene expression profiles of radioresistant cancers and cancer cell lines such as breast cancer, esophageal cancer and uterine cervical cancer were observed using microarray analysis (11-13).

With a focus on the prediction of radiosensitivity by using microarray analysis, this review summarizes preclinical and clinical data in both human cancer and cancer cell lines by comparing radiosensitive and radioresistant cell lines. To identify suitable publications, a search strategy was defined as follows. The Medline database was used by entering all possible combinations from one of the following key words; 'radiosensitivity/radiosensitive/radioresistant/radiation/radiotherapy,' with 'microarray'.

2. Microarray analysis comparing radiosensitive and radioresistant cancer samples and cancer cell lines

Presently, there are 17 reports comparing the gene expression profiles of radiosensitive and radioresistant cancer samples and cancer cell lines using microarray analysis. The site of

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these analyses are uterine cervical cancer (4 studies), head and neck cancer (3 studies), colorectal cancer (2 studies), breast cancer (1 study), esophageal cancer (1 study), pancreatic cancer (1 study), lung cancer (1 study), glioblastoma (1 study), hepatocellular carcinoma (1 study) and choroidal melanoma (1 study) (11-27).

Uterine cervical cancer. Kitahara *et al* compared gene expression profiles of 9 radiosensitive and 10 radioresistant tumors obtained by biopsy before treatment on a cDNA microarray consisting of 23,040 human genes (13). They identified 121 genes whose expression was significantly greater in radiosensitive cells than in radioresistant cells and 50 genes that reported higher levels of expression in radioresistant cells compared to radiosensitive cells. They discovered that genes involved in adipogenesis (*ALDH1*) and mitogen-activated protein kinase pathway (*MAP3K2*) were significantly upregulated in the responder group, while genes that are associated with the repair of breaks in double-strand DNA (*XRCC5*) were downregulated. The validity of the total 171 genes as being radiosensitivity-related genes was classified by the permutation test (p<0.05). Furthermore, they selected 62 genes on the basis of a clustering analysis and confirmed the validity of these genes with a cross-validation test. The cross-validation test also indicates the possibility of making a prediction of radiosensitivity for discriminating radiationsensitive from radiationresistant biopsy samples by predicting score values calculated from the expression values of 62 genes in 19 samples. Later, Harima *et al* conducted a subsequent follow-up analysis of *Ku80* levels (gene product of *XRCC5*) in 89 cervical cancer patients and they found that the low *Ku80* protein expression correlated well with sensitivity to radiation (28).

Additionally, Harima *et al* identified a set of genes related to thermoradiosensitivity of cervical cancer (14). They compared the expression profiles of 8 thermoradiosensitive and 11 thermoradioresistant tumors obtained by biopsy before treatment using a cDNA microarray consisting of 23,040 genes. They selected 35 genes on the basis of a clustering analysis and confirmed the validity of these genes with a cross-validation test. Some of these identified genes were associated with apoptosis such as BCL2-interacting killer (*BIK*), testis-enhanced gene transcript (TEGT), STAT induced STAT inhibitor 3 (*SSI-3*); hypoxia-inducible genes such as hypoxia-inducible factor 1 α (*HIF1A*), carbonic anhydrase XII (*CA12*) and some with tumor cell invasion and metastasis such as cathepsin L (CTSL), cathepsin B (*CTSB*), plasminogen activator urokinase (*PLAU*) and CD44 antigen (*CD44*).

In uterine cervical cancer, Tewari *et al* conducted a feasibility study of integrating an *in vitro* chemo-radiation response assay with a gene microarray system to investigate the molecular patterns of expression that contribute to radiation resistance in uterine cervical caner (15). Viable primary untreated cervical cancer specimens were obtained and exposed to γ irradiation at a dose of 3 Gy. Gene expression analysis was performed and the tumors were classified into extreme, intermediate and low radiation resistance categories. An intrinsic radiation response gene set of 54 gene transcripts with 100% accuracy for the classification of each tumor's radiation response category

was identified. They concluded that gene sets associated with *in vitro* radiation response profiles in cervical cancer can be generated using the *in vitro* chemo-radiation response assay and microarray technology.

Wong *et al* analyzed 16 patients with invasive squamous cell carcinoma of the uterine cervix, with total RNA extracted from tumor specimens, hybridized onto an oligonucleotide microarray consisting of over 20,000 transcripts (16). The genes were subjected to a statistical filter to identify statistically significant differential expression levels between those that were radiosensitive and those that were radioresistant. A backpropagation neural network was then constructed to model the differences so that patterns could be easily identified. Using the expression of the 10 most discriminating genes, they found that neural networks constructed from random subsets of the whole data were capable of predicting radiotherapy responses in the remaining subset, which appears stable within the dataset. They concluded that such an approach has the potential to differentiate tumor radiosensitivity, although confirmation of such a pattern using other larger independent datasets is necessary before a firm conclusion can be drawn.

Head and neck cancer. Higo *et al* conducted a microarray analysis of 2,201 genes using human oral squamous cell carcinoma cell lines (HSC2 as radioresistant and HSC3 as radiosensitive). They found 7 overexpressed genes in the radioresistant cell line and 2 over-expressed genes in the radiosensitive cell line (17). Of these, expression of T-complex protein 20 (*Tcp20*), which belongs to a family of molecular chaperones, was remarkably elevated in radioresistant HSC2 cells after irradiation. Other identified genes function as angiogenetic modulators such as heparin-binding protein 17 (*HBP17*) and catalysis of the cis to transisomerization (*Cyclophilin F*) was upregulated in radioresistant genes.

Ishigami *et al* compared gene expression signatures between oral squamous cell carcinoma cell lines exhibiting radioresistance and cells with radiosensitivity after X-ray irradiation in a dose-dependent manner using Affymetrix GeneChip analysis with Human Genome-U133 plus 2.0 GeneChip (18). The microarray data identified 167 genes that were significantly overexpressed in radioresistant cells after X-ray irradiation. Among the genes identified, 40 were mapped to 3 highly significant genetic networks identified by the Ingenuity Pathway Analysis tool. The 40 genes included 25 cancer-related genes that formed 1 network and were categorized by function into growth and proliferation, apoptosis and adhesion i.e., cyclooxygenase 2 (*COX-2*), intercellular adhesion molecule 2 (*ICAM2*), matrix metalloproteinase 13 (*MMP13*) and inhibitor of DNA binding 3 (*ID3*). They concluded that the identified genes help to elucidate the molecular mechanisms of the radioresistance of oral squamous cell carcinoma and can be radiotherapeutic molecular markers for choosing the appropriate radiotherapy for this disease.

Concerning clinical samples, Hanna *et al* utilized Atlas Human Cancer cDNA Expression Arrays to analyze the expression profiles of 1187 tumor-related genes in radiation-resistant and radiation-sensitive tissues (12). Sixty tumor-

related genes were selected as predictors of the radiation response of squamous cell carcinomas of the head and neck. These genes included some of the classically known radiation-responsive genes such as *c-jun* and *XRCC1*. Using the expression intensities of these 60 tumor-related genes, in combination with cluster analysis, they successfully predicted the radiation identity of two tumor samples.

Colorectal cancer. Ghadimi *et al* compared the global gene expression differences between radiation responders and non-responders with rectal cancer (19). Pre-treatment tumor biopsies from 30 patients who were randomized to receive radiotherapy and chemotherapy as part of the German Rectal Cancer Study (29) were studied and tumor response and a T-stage change. Using class comparison analysis between gene expression profiles from an initial run of 23 responsive or non-responsive tumors, 54 genes that were differentially expressed at significant levels were identified. Some of the genes are associated with DNA repair pathways (*SMC1*) and microtubule organization (CDC42 binding protein A [*cdc42BPA*], *Filamin B*). The ability to use these gene expression profiles to predict a response was then assessed using leave-one-out cross-validation. This generated an algorithm that allowed tumor behavior to be predicted with 78% sensitivity and 86% specificity. This algorithm was then further verified using a further 7 tumor samples hybridized on an alternative cDNA microarray platform which was able to correctly predict the response in 6 out of 7 patients. They concluded that pre-therapeutic gene expression profiling may assist in response prediction of rectal adenocarcinomas to preoperative chemoradiotherapy.

Watanabe *et al* constructed a new model using global gene expression profiling to predict the response to radiotherapy in rectal cancer (20). Using the human U95Av2 Gene Chip, they identified 33 novel discriminating genes of which the expression differed significantly between responders and non-responders. Using this gene set, they were able to establish a new model to predict responses to radiotherapy in rectal cancer with an accuracy of 82.4%. The list of discriminating genes included genes related to growth factors, apoptosis, cell proliferation, signal transduction and cell adhesion-related genes. The list of genes included the teratocarcinoma-derived growthfactor 3 (*TDGF3*), rho family member (*RAC2*), signal transduction protein tyrosine kinase (*TYRO3*) and cell proliferation phosphoserine phosphatase-like (*PSPHL*). Among the 33 discriminating genes, apoptosis inducers such as Lumican (*LUM*), thrombospondin 2 (*THBS2*) and galectin 1 (*LGALS1*) reported a higher expression in responders whereas apoptosis inhibitors such as cyclophilin 40 (*CYPD*) and glutathione peroxidase 2 (*GPX2*) showed higher expression in non-responders. The authors concluded that this model may be predicted to offer a new strategy for tailored therapy in rectal cancer.

Breast cancer. Helland *et al* analyzed the gene expression microarray analysis from the samples of 19 breast cancer patients before and after radiotherapy (2 Gy x 10 fractions) (21). They found that several genes involved in cell cycle regulation and DNA repair such as glutathione peroxidase 1 (*GPX1*); apoptosis, DNA binding protein 2 (*DDB2*); signal transduction, growth/differentiation factor 15 (*GDF15*); and cell cycle, cyclin-dependent kinase inhibitor 1A (*CDKN1A*) were significantly induced by radiation treatment. They also found that mutations were found in the *p53* gene in 39% of the tumors and the observed gene expression profiles seemed to be influenced by the *p53* mutation status.

Esophageal cancer. Fukuda *et al* established 4 radioresistant esophageal cancer cell lines by applying fractionated irradiation and compared the gene expression profiles of each parent and radioresistant line on cDNA microarrays consisting of 21,168 genes (11). They identified 19 up-regulated genes and 28 downregulated genes common to radioresistant sublines. Upregulated genes were associated with apoptosis and inflammatory responses baculoviral IAP repeat-containing 2 (*BIRC2*, *COX-2*), DNA metabolism (*CD73*) and cell growth (*PLAU*). Downregulated genes were associated with apoptosis such as caspase 6 (*CASP6*); cell adhesion (*CDH1*), transcription mixed-lineage leukemia 3 (*MLL3*); and cell cycle, cyclin-dependent kinase 6 (*CDK6*). Some of these genes were known to be associated with the radiation response, such as *COX-2*, but others were novel. They also examined the effect of radiotherapy on the expression of selected genes in clinical specimens. In 8 cases of squamous cell carcinoma of the esophagus, they found that the expression rates of BIRC2 for patients whose histological grade after radiotherapy (RT) was either 0 or 1 were significantly higher than those for patients whose histological grade after RT was either 2 or 3.

Pancreatic cancer. Ogawa *et al* established 5 radioresistant pancreatic cancer cell lines by applying fractionated irradiation and compared the gene expression profiles of each parent and radioresistant line on a oligonucleotide microarrays consisting of 17,086 genes (22). They identified 73 upregulated genes and 55 down-regulated genes common to radioresistant sublines. Upregulated genes were associated with growth factor signaling such as amphiregulin (*AREG*) and cell cycle checkpoints MAPK-activated protein kinase 2 (*MAPKAPK2*), intracellular signaling pathways (*regucalcin*) and angiogenic stimulation angiopoietin 2 (*ANGPT2*), whereas downregulated genes were associated with apoptosis such as caspase 8 (*CASP8*), retinoid esterification (*LRAT*) lecithin retinol acyltransferase and electron transport calcium-activated chloride 1 (*CACC1*). In particular, some of the identified genes such as *CASP8* and *MAPKAPK2* were known to be associated with a responsiveness to radiation.

Lung cancer. Guo *et al* compared 2 lung cancer cell lines with different radiosensitivity (NCI-H446 and A549, which is more radioresistant than NCI-H446) using oligonucleotide microarrays consisting of 143 genes (23). They found that the identified genes, such as *TP53,* were associated with apoptosis and cell proliferation, such as *MDM2* and *Bcl-2*, whose expression levels were increased in A549 (radioresistant) cells and decreased in NCI-H446 (radiosensitive) cells after irradiation. They also found that genes involved in DNA repair, such as *XRCC5* and the gene encoding DNA-PK, were found to be increased to a higher level in radioresistant A549 cells than in radiosensitive NCI-H446 cells.

Table I. Representative candidate genes related to radiosensitivity identified by microarray analysis.

Site (Ref.)	DNA-repair	Apoptosis	Growth factor	Signal transduction	Cell cycle	Cell adhesion	Invasion and metastasis	Angiogensis	Hypoxia -related	Others
Uterine cervix (13-16)	XRCC5	BIK SSI-3		MAP3K2			CTSL PLAU		HIF1A CA12	ALDH1
Head and neck (12,17,18)	XRCC1	ICAM2	COX2	c-JUN		MMP13 ID3		HBpl7		TCP20
Colon and rectum (19,20)	SMC1 THBS2	THBS2 GPX2	PSPHL TDGF3	RAC2 TYR03		CDC42BPA				
Breast (21)	DDB2	GPX1		GDF15	CDKN1A					
Esophagus (11)		CASP6 BIRC2	COX2	MLL3	CDK6	CDH1	PLAU			CD73
Pancreas (22)		CASP8	AREG	Regucalcin	MAPKAPK2 cyclin Al			AGPT2		LRAT
Lung (23)	XRCC5	TP53	MDM2 Bcl-2							
Glioblastoma (24)	XRCC5 G22P1									
HCC (25)	ERCC5 PMS1									
CLL (26)		c-myc		c-rel c-IAPl						HSP27
Choroidal melanoma (27)										ARPC1B

HCC, hepatocellular carcinoma; CLL, chronic lymphocytic leukemia.

Glioblastoma. Otomo *et al* analyzed transcriptional changes after ionizing radiation in 2 glioblastoma cell lines with different radiosensitivities (24). Between the 2 cell lines, the differences in the percentage of genes involved in DNA repair/replication was the largest and this category was present at a larger percentage in the radioresistant cell line. These genes included the *KRCC5* and *G22P1* (*Ku70*) genes, known to be important members of the non-homologous end-joining pathway of DNA double-strand break repair. They concluded that the distribution of genes represented in the DNA repair/replication category was most different between the two human glioblastoma cell lines of different radio-sensitivities.

Hepatocellular carcinoma. Jeoung *et al* evaluated the expression profiles of radiation-responsive genes in human hepatocellular carcinoma through time-dependent cDNA microarray analysis following exposure to ionizing radiation (25). In their study, DNA repair-promoting genes (such as *ERCC* and *PMS1*) were upregulated in radioresistant cells and downregulated or unchanged in radiationsensitive cells. They concluded that DNA repair capacity may control the radiation sensitivity of human hepatocelular carcinoma cells more than any other physiological factor.

B-cell chronic lymphocytic leukemia. Vallat *et al* (26) conducted a microarray analysis of the responses of B-cell chronic lymphocytic leukemia cells that were both sensitive (4 samples) and resistant (3 samples) to radiation. Sixteen genes were differentially regulated by at least 2-fold specifically in the resistant cells and they confirmed their altered expression levels in 15 additional B-cell chronic lymphocytic leukemia samples not included in the microarray analysis. These identified genes included *c-myc*, *c-rel*, and *c-IAP1*; 2 of these genes, *c-rel* and *c-IAP1*, have direct connections to the NF-κB pathway (30). Resistant cells also had downregulated genes, including *stat1* and heat shock protein 27 (*HSP27*), in spite of differences in *p53* status. These results suggest that these differentially expressed genes may be of clinical relevance and can also be used as prognostic indicators in patients with B-cell chronic lymphocytic leukemia.

Malignant melanoma. Kuamgai *et al* analyzed choroidal malignant melanoma cell lines using an oligonucleotide microarray containing 10,800 genes (27). They found 34 genes that were differentially expressed in the OCM-1 and OCM-2 cell lines (radioresistant) compared with the 92-1 cell line (radiosensitive). They selected 13 genes that were

confirmed by RT-PCR and investigated the correlation between gene expression and SF2 (surviving fraction at 2 Gy irradiation) using Pearson's correlation coefficient and the Fisher's Exact test on the 13 genes. Of these genes, the actin polymerization-related gene actin related protein 2/3 complex 41kd (*Arpc1b*) was found to be a candidate predictive marker for radiation sensitivity of choroidal malignant melanoma.

3. Summary of the identified genes from cancer cell lines investigated by microarray

These reports have indicated that the majority of the identified genes relating to radiosensitivity in human cancer have been associated with apoptosis, DNA-repair, growth factor, signal transduction, cell cycle and cell adhesion, invasion and metastasis, angiogenesis and hypoxia (Table I). Some of the genes, such as *BIRC2*, were to be a predictor of the radiation response in human cancer. Moreover, several groups demonstrated that gene analysis is able to predict the tumor response to radiotherapy in several cancer types.

In conclusion, this review, along with previous reports, indicates that genes related to radiosensitivity are frequently associated with DNA repair, apoptosis, growth factor, signal transduction, cell cycle and cell adhesion. Therefore, these genes can contribute to radioresistance in many cancer types. Our review also confirmed that several genes were found to be predictors of the radiation response with various cancers and certain sets of identified genes were also found to predict the radiation response by using clustering analysis. Analyses of global gene expression profiling between radiation responders and non-responders can be useful in predicting the response to radiotherapy and can also provide insights into the mechanism and development of individual therapies and novel therapeutic targets.

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