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Systematic Analyses of Genes Associated with Radiosensitizing Effect by Celecoxib, a Specific Cyclooxygenase-2 Inhibitor

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COX-2/Celecoxib/siRNA/cDNAmicroarray/Ionizing radiation.

To investigate genes regulated by COX-2 or a COX-2 specific inhibitor, celecoxib, in irradiated cancer cells, we analyzed changes in gene expression using complementary DNA microarray following celecoxib or combined celecoxib and ionizing radiation (IR) treatment in a stable COX-2 knockdown A549 (AS) and a mock cell line (AN). Thirty-six genes were differentially expressed by COX-2 knockdown. Celecoxib changed the expressions of 40 and 69 genes in AN and AS cells, respectively. Twenty-seven genes were synchronously regulated by COX-2 and celecoxib. Among these, celecoxib regulated ras homolog gene family B and mitosin protein expression in a COX-2 dependent manner, especially in irradiated cells. In addition, we identified 11 genes that changed by more than 1.5 times the expected additive values after celecoxib and IR treatment. The current study may provide evidence that COX-2 or celecoxib regulates various intracellular functions in addition to their enzymatic activity regulation. We also identified candidate molecules that may be responsible for COX-2-dependent radiosensitization by celecoxib.

INTRODUCTION

Cyclooxygenase (COX) is an enzyme that produces various prostanoids. COX is known to have two isoforms, COX-1 and COX-2.[1)] COX-1 is constitutively expressed in most tissues and synthesizes various prostaglandins that control normal physiologic functions and maintain homeostasis.[1–3)] In contrast, COX-2 is not detected in most normal tissues, being generally induced by various stimuli (pro-inflammatory factors, growth factors, cytokines and oncogenes), although recent studies report constitutively COX-2 expression for specific normal functions such as neurotransmission, reproduction, and renal physiology.[1)]

Nearly 40% of colon adenomas and about 90% of adenocarcinomas originating from various organs including breast, lung, prostate, or cervix overexpress COX-2.[4)] In addition, high levels of prostaglandin E_2 (PGE_2), a major product of COX-2, are detected in a number of human tumors, but not in surrounding normal tissues. Increased PGE_2 expression is known to promote cell motility, proliferation, invasion, and angiogenesis, while inhibiting apoptosis and immune surveillance.[5–7)] These functions seem to be related to cancer development. Therefore, COX-2 and its gene products have been considered important therapeutic targets for cancer treatment.[8,9)]

Celecoxib, a COX-2 specific inhibitor, is a non-steroidal anti-inflammatory drug (NSAID) approved by the United States Food and Drug Administration (FDA) to prevent colon cancer in patients with familial adenomatous polyposis syndrome.[2,5,10)] Celecoxib is a water insoluble drug and, when administered orally in several clinical trials, reached a maximum plasma concentration of 3 to 10 μM. Celecoxib has been shown to reduce the number of colorectal polyps by about 28%.[11,12)] Recent data using genomic and proteomic tools have also shown that celecoxib regulates genes associated with anticancer functions such as growth arrest or apoptosis induction in COX-2-dependent or -independent manners.[2,6,9,13,14)] This evidence suggests that COX-2 is intimately related to the genetic changes involved in carcinogenesis and tumor growth, and that modulating intracellular COX-2 or administering celecoxib can alter COX-2-dependent or -independent cancer-related target genes.[5,8)]

We recently reported that celecoxib radiosensitizes cancer cells in a COX-2 dependent manner.[15)] However, the underlying molecular mechanisms are still poorly understood. In

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order to understand the genes regulated by COX-2 expression and to infer genes and signaling networks associated with the radiosensitizing effects of celecoxib,[15,16] we analyzed genetic changes using complementary DNA (cDNA) microarrays after a COX-2 siRNA knockdown or celecoxib administration, with or without ionizing radiation (IR) exposure, in a constitutive COX-2-overexpressing A549 cancer cell line.

MATERIALS AND METHODS

Cell culture and treatment

A549 lung adenocarcinoma cells and HCT-116 human colon adenocarcinoma cells were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). COX-2 knock-down A549 cells (AS) and their mock control cells (AN) were developed using COX-2 siRNA as previously described.[17] AN and AS stable cells were maintained in medium containing 350 μg/mL G418 (GibcoBRL, USA). Stable COX-2–overexpressing cell line (HCT116–COX-2) and mock vector-transfected control cell line (HCT116–Mock) were developed from HCT116 parent cells as described previously,[18] and they were maintained in a medium containing 100 μg/mL hygromycin B (Invitrogen). Cells were grown up to 80% confluence in 75 cm^2 T-flasks. Cells were treated with 50 μM celecoxib (provided by Pfizer, USA) for 24 h with or without exposure to 6 Gy IR, and then harvested after washing twice with ice-cold PBS. Final DMSO concentration in culture media was maintained below 0.1%.

Human cDNA microarray design

We designed cDNA microarray experiments as shown in Fig. 1 to analyze genes regulated by COX-2 or its specific inhibitor, celecoxib, before and after IR-exposure in cancer cells. A common reference RNA pool was prepared using Universal Human Reference RNA (Stratagene, La Jolla, CA) as an internal reference standard. Microarrays were performed to compare each sample with this common reference, allowing for cross-comparisons of multiple data sets from different conditions.

cDNA probe preparation and microarray hybridization

Total cellular RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. The common reference RNA was prepared by combining equal amounts (100 μg) of total RNA isolated from AN and AS cells. The cDNA microarray contained 17,448 sequence-verified human cDNA clones was performed by GenomicTree, Inc (Daejeon, Republic of Korea). Target cDNA probe synthesis and hybridization were performed as previously described.[19] One hundred micrograms total RNA was reverse-transcribed in the presence of Cy^3 or Cy^5-dUTP (NEN Life Sciences) at 42°C for 2 h. Common reference RNA was labeled with fluorescent Cy^3-dUTP and total RNAs from indicated conditions were labeled with fluorescent Cy^5-dUTP. Both Cy^3 and Cy^5-labeled cDNA were purified using a PCR purification kit (Qiagen) as recommended by the manufacturer. The purified cDNA was resuspended in 80 μL of hybridization solution containing 3.5X SSC, 0.3% SDS, 20 μg of human Cot-1 DNA, 20 μg of poly A RNA and 20 μg of yeast tRNA (Invitrogen). The hybridization mixtures were heated at 100°C for 2–3 min and directly pipetted onto microarrays. The arrays hybridized at 65 for 12–16 h in a humidified hybridization chamber. The hybridized microarrays were washed with 2 × SSC for 2 min, 0.1 × SSC/0.1% SDS for 5 min, and 0.1 × SSC for 5 min. The washed microarrays were immediately dried using a microarray centrifuge, and fluorescence signals were acquired using a GenePix 4000B laser scanner (Axon Instrument Inc, Union City, CA). All microarray hybridizations were performed in duplicate and the data were averaged. The pearson's correlation coefficient (r^2) was calculated using $\log_2$ ratio to assess the reproducibility between two technically replicated microarray experiments and was > 0.9. Only the data that was showed the same effect has been selected for analysis.

Data acquisition and analysis

Hybridization images were analyzed by GenePix Pro 4.0 (Axon Instruments, CA). The average fluorescence intensity for each spot was calculated and the local background was subtracted. All data normalization and statistical analyses were performed using GeneSpring 6.1 (Silicon Genetics, USA). Genes were filtered according to the two-component model for estimating variation from the control strength.[20] Intensity-dependent normalization (LOWESS) was performed, where the ratio was reduced to the residual of the Lowess fit of the intensity vs. ratio curve. The averages of the normalized ratios were calculated by dividing the average normalized signal channel intensity by the average normalized control channel intensity. The ANOVA test (parametric) was performed using a Benjamin and Hochberg false discovery rate correction at p values < 0.01 to find differentially expressed genes across samples. Hierarchical clustering was performed by similarity measurements based on Pearson correlations around 0. Functional annotations of genes were performed using AmiGO (http://amigo.geneontology.org/cgi-bin/amigo/go.cgi).

Reverse transcription-PCR (RT-PCR)

For RT-PCR, total RNA (2 μg) was reverse transcribed for 1 h at 42°C in a reaction mixture (Clontech., USA) that contained 1 mmol/L deoxynucleotide triphosphate, 2.5 μmol/L oligo $(dT)_{18}$, 8 mM DTT, 0.4 mg/mL BSA, 1 × reverse transcriptase buffer, and 1 μg powerscript reverse transcriptase. We conducted PCR for *COX-2*, *Midline 1*, *Mitosin*, *Phsopholipase A2*, *Carbonic anhydrase XII* (*CA12*), and *ras homolog gene family B* (*Rho B*) in a PCR machine (GeneAmp

PCR System 9700, Applied Biosystems). Glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was used as an internal control.

Western blot analysis

Western blot analysis was performed as previously described.[21] Cells (AN/ AS and HCT116-Mock/-COX-2) were treated with 50 μM celecoxib ± 12 Gy IR and then after 24 h, the cells were harvested. The cells were lysed on ice with lysis buffer (10 mM Tris-Cl, pH 8.0, 100 mM NaCl, 1% Triton X-100, and 1 mM EDTA) containing 10 μg/mL aprotinin, 10 μg/mL leupeptin, 1 μg/mL pepstatin, 100 μg/mL phenylmethylsulfonyl fluoride, 10 mM NaF, and 10 mM Na_3VO_4 for 15 minutes. Proteins (30 μg) were separated by SDS-PAGE, transferred to PVDF membranes, and probed with the following antibodies: a monoclonal antibody to COX-2 (BD Transduction, USA), a monoclonal antibody to RhoB (Santa Cruz Biotechnology, Santa Cruz, CA, USA), and a monoclonal antibody to Mitosin (BD Transduction). The membranes were also re-probed with a monoclonal β-actin antibody (Sigma) to normalize loading differences between the samples. All experiments were performed at least in triplicate.

RESULTS

The differentially expressed genes in stably COX-2 knockdown cells

To analyze genes regulated by COX-2 or celecoxib before and after IR-exposure in cancer cells, we designed human cDNA microarray experiments as shown in Fig. 1A. We selected A549 lung cancer cells, which constitutively express a high level of COX-2 and developed a stable COX-2 knockdown cell line (AS) using a vector expressing COX-2 specific siRNA. COX-2 expression in AS cells stablely decreased to about $1/30^{th}$ that of the AN cells (Fig. 1B).

First, we analyzed genetic changes induced by COX-2 knockdown. We found that 36 genes were differentially expressed (a common set of genes that changed > two-fold and genes changed significantly by ANOVA test) in AS cells compared to AN cells (Table 1). The ratio of AS/AN mRNA expression in Table 1 shows that COX-2 knockdown downregulated 12 of 36 genes and upregulated 24 genes. When classified by biological functions, the differentially expressed genes by COX-2 knockdown were found to be related to enzymes (13 genes), signal transduction (six genes), transporter (four genes), cell cycle (two genes), and nucleic acid binding (two genes). COX-2 knockdown was shown to modulate many genes encoding enzymes that are related to oxidation/reduction and transporters. Of note, COX-2 knockdown downregulated fibroblast growth factor receptor and upregulated CA12, suggesting that COX-2 may be involved in angiogenesis- and hypoxia-regulatory pathways. COX-2 knockdown downregulated the expression levels of the cell cycle-related genes, mitosin and midline 1. Mitosin is a centromere protein in the kinetocore and is associated with cell cycle regulatory functions such as mitosis, G_2-M transition, and the spindle assembly checkpoint.[22] Midline 1 acts on microtubule anchors, cytoskeleton organization, and chromosome segregation during mitosis.[23]

These findings imply that COX-2 may be associated with various intracellular functions including energy metabolism, angiogenesis, hypoxia-regulation (oxidation/reduction), and cell cycle regulation in cancer cells.

Functional classification of genes modulated by celecoxib

Next, we analyzed genetic changes following celecoxib treatment of AN or AS cells using the same analytical method shown previously. We found that 40 and 69 genes changed expression following celecoxib treatment in AN (Table 2) and AS cells (Supplementary Table 1), respectively. About three-fourths of the total genes (74 and 73%) regulated by celecoxib were involved in signal transduction, enzymes, cell cycle regulation and nucleic acid binding in both AN and AS cells (Fig. 2A). These results suggest that celecoxib modulates many genes involved in various intracellular functions in both COX-2 over- and low-expressing cells. Among these celecoxib-regulated genes, eight genes (cell division cycle 2 , cyclin D3, midline 1, ZW10 interactor, tubulin 5, cysteine-rich angiogenic inducer, DNA damage-inducible protein, and spermine oxidase) were common to both AN and AS cells (Fig. 2B). These genes may be regulated by celecoxib in a COX-2-independent manner. Notably, four of eight genes (cell division cycle 2, cyclin D3, midline 1, ZW10 interactor) are related to cell cycle regulation, suggesting that celecoxib may intimately regulate cell cycle in a COX-2-independent manner.

In addition, we found that celecoxib downregulated expression of heat shock protein 70 (hsp70) and upregulated an apoptosis-related genes, prostate apoptosis response-4 protein (Par-4) only in AS cells (Supplementary Table 1). These results suggest that celecoxib may also regulate a chaperone system and may induce apoptosis using Par-4-related pathways in COX-2-dependent manners.

Selection of synchronously regulated genes by celecoxib and COX-2 expression to find responsible molecules for COX-2 dependent radiosensitization by celecoxib

We found 158 genes that were up- or downregulated more than two-fold in AS cells compared to AN cells. Expression of 364 genes changed more than two-fold following celecoxib treatment in AN cells. Twenty-seven genes were common to these two groups, indicating that these genes are synchronously regulated by COX-2 and celecoxib (Fig. 3A). Celecoxib may regulate some of these genes in a COX-2-expression-dependent manner and therefore may contain

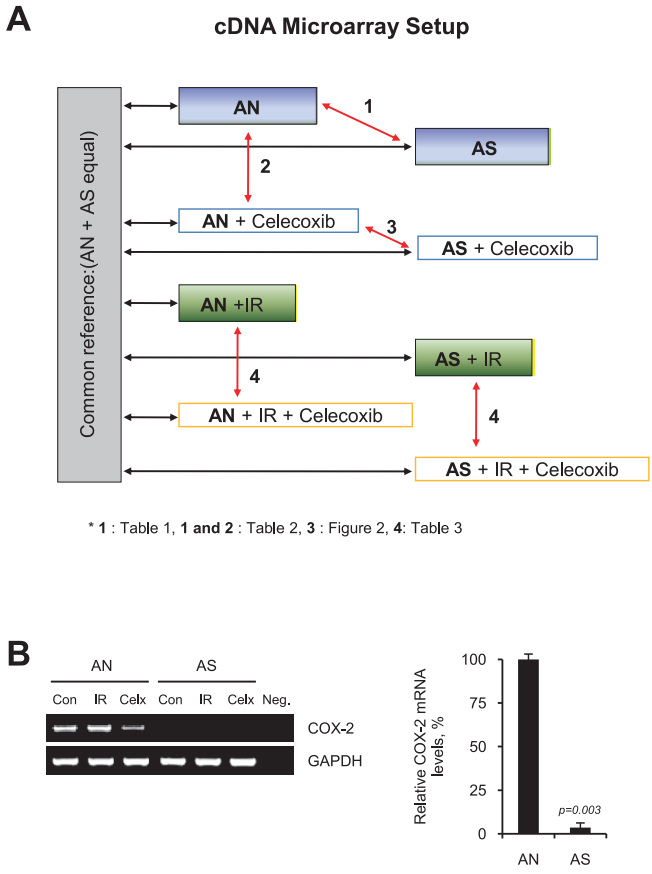

Fig. 1. cDNA microarray setup to determine differentially regulated genes by celecoxib and IR in AN and AS cells. **A.** cDNA microarray experiment was designed to analyze genes regulated by COX-2 or celecoxib, before and after IR exposure, in AN or AS cells. A common reference RNA pool was prepared and comparisons were performed between the common reference and each group to allow for cross-comparisons of multiple data sets from different conditions. AN; A549-Mock cells, AS; A549-COX-2 knockdown cells. **B.** COX-2 expression levels of AN and AS cells were confirmed by RT-PCR and quantified using Multi Gauge V3.0 program. All experiments and measurements were done at least in triplicate. Con; 0.1% DMSO control, IR; 6 Gy irradiation, Celx; 50 μM Celecoxib, Neg.; negative control.

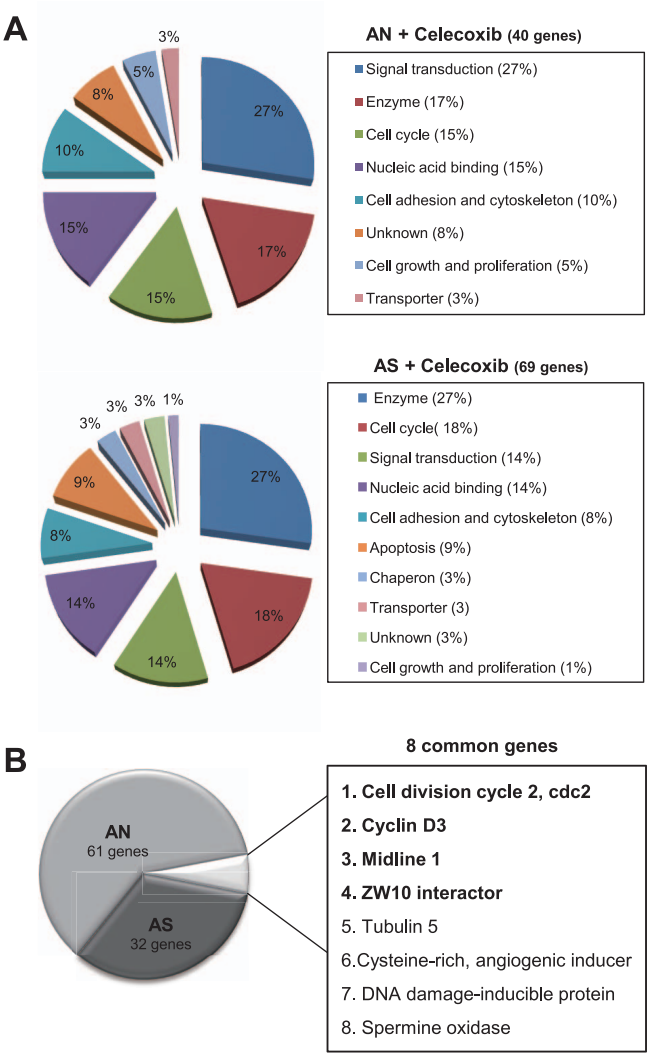

Fig. 2. Functional classification of genes changed by celecoxib in AN and AS cells. **A.** AN and AS cells were treated with or without 50 μM celecoxib for 24 h and then defined significantly changed genes in each cells. The percent in each functional category represents the proportion of genes involved in the indicated category among the total changed genes. **B.** The list of genes common to both AN and AS cells among the celecoxib-regulated genes.

genes responsible for COX-2-dependent radiosensitization by celecoxib that we have previously shown.[15] These genes could be COX-2 activity-dependent genes (i.e., genes dependent on product of COX-2) but could also be COX-2-expression-dependent but COX-2 activity-independent genes since we observed COX-2-expression-dependent radiosensitization by celecoxib that was not reversed by administration of PGE_2, a major product of COX-2.[15] COX-2 has peroxidase activity as well as PG-producing cyclooxygenase activity, and celecoxib seems to have other functions that are COX-2-expression dependent but cyclooxygenase-activity independent.[15]

We analyzed these 27 genes further. COX-2 knockdown and celecoxib synchronously downregulated 18 genes (group 1), one gene was downregulated by COX-2 knockdown but upregulated by celecoxib (group 2), and COX-2 knockdown and celecoxib synchronously upregulated eight genes (group 3) (Table 3 and Fig. 3B).

Genes in group 1 were related to cell cycle regulation (M-phase phosphoprotein 1, mitosin, CCDC28B coiled-coil domain containing 28B, midline 1), growth and proliferation (bone morphogenetic protein 4, phospholipase A_2, G protein, neurotensin), and cell adhesion (junctional adhesion molecule 3). In contrast, genes in group 3 were related with apoptosis (melanophilin, TGF-β, RhoB), glycolysis (aldolase

Table 1. Genes changed by COX-2 knockdown in AS cells compared to AN cells (36 genes).

	Accession No.	Symbol	Common Name	AN	AS	Ratio (AS/AN)
Down	***Signal transduction***					
(12 genes)	AA281729	ARL8	ADP-ribosylation-like factor 8	1.41	0.41	0.29
	AA281064	FGFR1	fibroblast growth factor receptor 1	1.15	0.40	0.34
	AI356712	RPGR	retinitis pigmentosa GTPase regulator	1.39	0.52	0.38
	Cell cycle					
	AA701455	CENPF	centromere protein F, 350/400 ka (mitosin)	0.73	0.29	0.40
	AA598640	MID1	midline 1 (Opitz/BBB syndrome)	0.91	0.44	0.48
	Enzyme					
	AA775223	HPGD	hydroxyprostaglandin dehydrogenase 15-(NAD)	1.29	0.45	0.35
	AA481052	ZDHHC14	zinc finger, DHHC domain containing 14	0.98	0.39	0.40
	Apotosis					
	AW029497	SLC18A2	solute carrier family 18 (vesicular monoamine), member 2	1.31	0.50	0.39
	Nucleic acid binding					
	AA455237	IRAK1BP1	interleukin-1 receptor-associated kinase 1 binding protein 1	1.16	0.46	0.39
	Unknown					
	AA280514		zt09f05.s1 NCI_CGAP_GCB1 Homo sapiens cDNA clone	1.14	0.26	0.22
	AA933025		oo42h06.s1 NCI_CGAP_Lu5 Homo sapiens cDNA clone	2.29	0.56	0.24
	AA489619		aa43a05.s1 Soares_NhHMPu_S1 Homo sapiens cDNA clone	1.67	0.60	0.36
Up	***Signal transduction***					
(24 genes)	R62817	STOM	stomatin	0.65	1.50	2.30
	AA875933	EFEMP1	EGF-containing fibulin-like extracellular matrix protein 1	0.59	1.88	3.18
	AI308789	EFHD2	EF-hand domain family, member D2	0.73	2.32	3.19
	Enzyme(oxidation and reduction)					
	AA486275	SERPINB1	serine (or cysteine) proteinase inhibitor, clade B (ovalbumin), member 1	0.42	1.02	2.41
	AA876375	LTB4DH	leukotriene B4 12-hydroxydehydrogenase	0.41	1.34	3.27
	AI986336	CA12	carbonic anhydrase XII	0.15	0.55	3.68
	R91950	CYB5	cytochrome b-5	0.40	1.57	3.93
	AI540460	PTGES	prostaglandin E synthase	0.36	1.50	4.19
	AA156988	ACO1	aconitase 1, soluble	0.27	1.32	4.89
	AA436163	PTGES	prostaglandin E synthase	0.32	1.59	5.00
	AA701963	AKR1B1	aldo-keto reductase family 1, member B1 (aldose reductase)	0.21	1.35	6.52
	AA916325	AKR1C3	aldo-keto reductase family 1, member C3	0.21	1.97	9.37
	AI924753	AKR1B10	aldo-keto reductase family 1, member B10 (aldose reductase)	0.06	2.17	36.92
	AI301329	AKR1B10	aldo-keto reductase family 1, member B10 (aldose reductase)	0.06	4.36	69.74
	Transporter					
	AA490962	KIAA1228	extended synaptotagmin-like protein 2	0.69	1.41	2.05
	T62040	ETFB	electron-transfer-flavoprotein, beta polypeptide	0.57	1.44	2.54
	R26732	PMP22	peripheral myelin protein 22	0.69	1.76	2.55
	AA634267	NPC1	Niemann-Pick disease, type C1	0.48	1.59	3.31
	Cell growth and proliferation					
	W73810	EMP3	epithelial membrane protein 3	0.41	3.19	7.80
	AA873604	CRIP1	cysteine-rich protein 1 (intestinal)	0.16	3.35	20.89
	Cell adhesion					
	AA485677	TRIP6	thyroid hormone receptor interactor 6	0.69	1.84	2.68
	Nucleic acid binding					
	H96235	ETS2	v-ets erythroblastosis virus E26 oncogene homolog 2 (avian)	0.14	1.86	13.51
	Unknown					
	H59916		yr04f12.s1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone	0.55	1.43	2.61
	T62048		yc66c02.s1 Stratagene liver (#937224) Homo sapiens cDNA clone mRNA seq.	0.23	1.30	5.64

Accession number refers to GeneBank accession number. The information on the gene common name and gene symbol were from SOURCE (http://source.stanford.edu). If an AS/AN ratio is below 1, it is referred to as downregulated, or if above 1, as upregulated.

Table 2. Genes changed following celecoxib treatment in AN cells (40 genes).

Accession No.	Symbol	Common Name	Control	Celecoxib
Signal transduction				
R16073	INSL4	insulin-like 4 (placenta)	1.00	0.16
AA425401	STK24	serine/threonine kinase 24 (STE20 homolog, yeast)	1.00	0.30
AA454098	KIF23	kinesin family member 23	1.00	0.28
AA463225	BMP4	bone morphogenetic protein 4	1.00	0.30
AI984983	PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)	1.00	0.31
AA999901	GNG11	guanine nucleotide binding protein (G protein), gamma 11	1.00	0.33
R45054	CRH	corticotropin releasing hormone	1.00	0.35
AA454868		Homo sapiens transcribed sequence with moderate similarity to protein ref:NP_006198.1 (H.sapiens) platelet-derived growth factor receptor-like protein; platelet-derived growth factor-beta-like tumor suppressor [Homo sapiens]	1.00	0.36
AA431321	HMGN3	high mobility group nucleosomal binding domain 3	1.00	0.36
AA953249	HF1	H factor 1 (complement)	1.00	0.40
N48319	BCAR3	breast cancer anti-estrogen resistance 3	1.00	2.11
Cell cycle				
AA278384	CDC2	cell division cycle 2, G1 to S and G2 to M	1.00	0.21
AA706968	ZWINT	ZW10 interactor	1.00	0.27
R46787	CCNB1	cyclin B1	1.00	0.33
AI340905	CCND3	cyclin D3	1.00	0.37
AA283006	SMC4L1	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)	1.00	0.39
AA598640	MID1	midline 1 (Opitz/BBB syndrome)	1.00	0.45
Enzyme				
AA456621	GGH	gamma-glutamyl hydrolase (conjugase, folylpolygammaglutamyl hydrolase)	1.00	0.36
AA115877	SERPINI1	serine (or cysteine) proteinase inhibitor, clade I (neuroserpin), member 1	1.00	0.37
AW082097	PI3	protease inhibitor 3, skin-derived (SKALP)	1.00	0.40
AI352323	FIGNL1	fidgetin-like 1	1.00	0.45
AA485743	C9orf3	chromosome 9 open reading frame 3	1.00	0.46
AI871665	ACAT1	acetyl-Coenzyme A acetyltransferase 1 (acetoacetyl Coenzyme A thiolase)	1.00	0.49
H93328	SMOX	spermine oxidase	1.00	5.85
Nucleic acid binding				
AA418045	RFX5	regulatory factor X, 5 (influences HLA class II expression)	1.00	0.38
AA461098	LSM3	LSM3 homolog, U6 small nuclear RNA associated (S. cerevisiae)	1.00	0.39
AI985549	SRP46	Splicing factor, arginine/serine-rich, 46 kD	1.00	0.45
AI375411	SOX2	SRY (sex determining region Y)-box 2	1.00	0.50
AW072780	EIF4G1	eukaryotic translation initiation factor 4 gamma, 1	1.00	2.41
T67270	RPL10	ribosomal protein L10	1.00	3.91
Cell adhesion and cytoskeleton				
H63096	HLA-DMB	major histocompatibility complex, class II, DM beta	1.00	0.30
AA479933	LOC51668	HSPCO34 protein	1.00	0.35
N74524	TUBB5	tubulin, beta, 5	1.00	0.44
AA176957	NEB	nebulin	1.00	0.44
Transporter				
N52267	AP2B1	adaptor-related protein complex 2, beta 1 subunit	1.00	4.41
Cell growth and proliferation				
AA777187	CYR61	cysteine-rich, angiogenic inducer, 61	1.00	2.57
AA015892		similar to gb:S62138 growth arrest and DNA -damage-inducible protein GADD153	1.00	2.96
Unknown				
AA454572		zx74g06.s1 Soares ovary tumor NbHOT Homo sapiens cDNA clone	1.00	0.30
AW073291	AGR2	anterior gradient 2 homolog (Xenopus laevis)	1.00	0.37
AA169645		zo94b07.s1 Stratagene ovarian cancer (#937219) Homo sapiens cDNA clone	1.00	0.40

Genes that changed > two-fold in both direct and indirect comparisons and by ANOVA test were selected.

Table 3. Synchronously regulated genes by COX-2 knockdown and celecoxib (27 genes).

	Accession No.	Symbol	Common Name	AN	AS/AN	AN + Celx/ AN	Up (+) or Down (–)
Group 1	***Cell cycle and chromosome related genes (mitosis)***						
(18 genes)	AA214392	SMC2L1	SMC2 structural maintenance of chromosomes 2-like 1 (yeast)	1	0.26	0.34	_
	AA283006	SMC4L1	SMC4 structural maintenance of chromosomes 4-like 1 (yeast)	1	0.34	0.40	_
	AA282936	MPHOSPH1	M-phase phosphoprotein 1	1	0.35	0.40	_
	AA701455	CENPF	centromere protein F, 350/400ka (mitosin)	1	0.42	0.45	_
	AA975458	MGC1203	CCDC28B coiled-coil domain containing 28B	1	0.33	0.46	_
	Signal transduction (growth and proliferation)						
	AA463225	BMP4	bone morphogenetic protein 4	1	0.25	0.30	_
	AI984983	PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)	1	0.11	0.31	_
	AA999901	GNG11	guanine nucleotide binding protein (G protein), gamma 11	1	0.38	0.33	_
	AA953249	HF1	H factor 1 (complement)	1	0.31	0.40	_
	AI270779		similar to SW:NEUT_HUMAN P30990 nurotensin/nuromedin N precursor	1	0.03	0.45	_
	AA928656	NTS	neurotensin	1	0.01	0.47	_
	Cell adhesion and Cytoskeleton						
	AA918982	HPS3	Hermansky-Pudlak syndrome 3	1	0.28	0.40	_
	AA931102	JAM3	junctional adhesion molecule 3	1	0.29	0.40	_
	AA598640	MID1	midline 1 (Opitz/BBB syndrome)	1	0.48	0.45	_
	H63096	HLA-DMB	major histocompatibility complex, class II, DM beta	1	0.14	0.30	_
	Transporter						
	AA262080	SLC12A2	solute carrier family 12 (sodium/potassium/chloride transporters), member 2	1	0.43	0.49	_
	Unknown						
	AA933025	AI733274	oo42h06.s1 NCI_CGAP_Lu5 Homo sapiens cDNA clone	1	0.27	0.25	_
	AI27779		unknown	1	0.02	0.49	_
Group 2	H59916		yr04f12.s1 Soares fetal liver spleen 1NFLS Homo sapiens cDNA clone	1	2.73	0.49	±
Group 3	***Signal transduction (apotosis)***						
(8 genes)	AA976544	MLPH	melanophilin	1	3.44	2.02	+
	AA495790	ARHB	ras homolog gene family, member B	1	4.95	2.07	+
	AI365418	BRE	brain and reproductive organ-expressed (TNFRSF1A modulator)	1	2.50	2.46	+
	R36467		similar to gb:X02812_cds1 transforming growth factor beta 1 precursor	1	3.44	2.80	+
	Transporter						
	AA630794	SLC3A2	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	1	3.40	4.09	+
	Enzyme (gycolysis)						
	AA775241	ALDOA	aldolase A, fructose-bisphosphate	1	4.70	3.42	+
	AA171613	FLJ20151	Carbonic anhydrase 12	1	4.26	2.07	+
	Nucleic acid binding						
	AW072780	EIF4G1	eukaryotic translation initiation factor 4 gamma, 1	1	6.96	2.41	+

AS/AN ratio refers to changes of genes in AS cells compared to AN cells. AN + Celx/AN ratio refers to changes of genes in celecoxib treated AN cells compared to untreated AN cells. When both AS/AN and AN + Celx/AN ratios were below 1, it is referred to as "down (–)", when both were above 1, it is referred to as "up (+)".

A, CA12), and nucleic acid binding (eukaryotic translation initiation factor 4). These results suggest that these 27 genes are associated with functions that could be important for cancer cell growth and survival. One or more of these genes may be related to the molecular mechanisms underlying COX-2-dependent radiosensitization by celecoxib.

Validation of genes related to radiosensitizing effect by celecoxib among synchronously regulated genes by celecoxib and COX-2 expression

To validate genes associated with celecoxib induced radiosensitizing effect dependent or independent on COX-2 in cancer cells, we selected five genes among the 27 genes

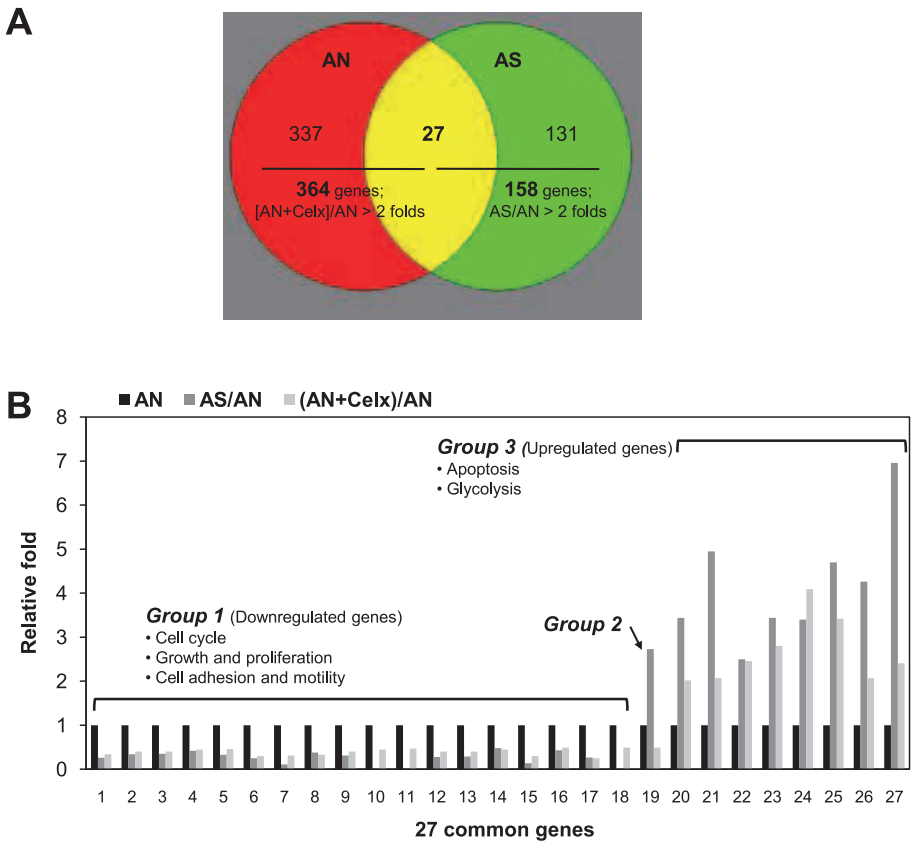

Fig. 3. Synchronously regulated genes by COX-2 knockdown and celecoxib. Microarrays to define genetic changes > two-fold by COX-2 knockdown were done using AN and AS cells. Microarrays to define genetic changes > two-fold by celecoxib treatment were done in AN cells after treatment with 50 μM celecoxib for 24 h. A common set of these two microarray data was identified to define genes that are synchronously regulated by COX-2 knockdown and celecoxib. **A.** Venn diagrams show the number of changed genes following celecoxib treatment in AN (364 genes) or AS (158 genes) cells. Twenty seven genes were common to both cells. **B.** Expression values of the 27 genes in AS or celecoxib-treated AN cells were normalized by those in AN cells.

synchronously regulated by COX-2 and celecoxib, which were associated with cell cycle regulation (mitosin, RhoB, midline) and tumorigenesis (phospholipase A2 and CA12). To futher verify changes of these genes by COX-2 and celecoxib, AN and AS cells were treated with vehicle or 50 μM celecoxib for 24 h, and mRNA levels were analyzed by RT-PCR (Fig. 4A). Among group 1 genes, mRNA expressions of midline 1, mitosin, and phospholipase A_2 decreased in AS cells compared to AN cells, and also decreased following celecoxib administration in AN cells (Fig. 4A). These results are consistent with the cDNA microarray data. As described previously, mitosin and midline 1 are associated with cell cycle regulation. Phospholipase A_2 releases arachidonic acid, the main COX-2 substrate, from the cell membrane.

In group 3 genes, the CA12 mRNA level increased in AS cells compared to AN cells and further increased following celecoxib treatment in both AN and AS cells (Fig. 4B). RhoB mRNA increased in AS cells compared to AN cells, and showed almost no change following celecoxib administration in AN cells, but was decreased significantly in AS cells (Fig. 4B). These results are similar to those of the microarray data. CA12 is a membrane zinc metalloenzyme that catalyzes the reversible CO_2 hydration to form bicarbonate, thereby regulating the microenvironment acidity and tumor malignant phenotype. CA12 is present in a variety of normal tissues but is overexpressed in cancer cells. Therefore, CA12 seems to be associated with tumorigenesis.[24] RhoB is a Ras-related GTPase that provides negative cell cycle regulation. Farnesylated RhoB inhibits IR-induced mitotic cell death, increases G_2 arrest, and controls IR-induced centrosome overduplication.[25] Several papers have reported an interaction between RhoB and COX-2, and it is also known to be involved in regulation of radiosensitivity.[26]

Mitosin and Rho B may be candidate molecules for radiosensitization by celecoxib

We previously reported that celecoxib radiosensitizes cancer cells in a COX-2 dependent manner, and we also recently

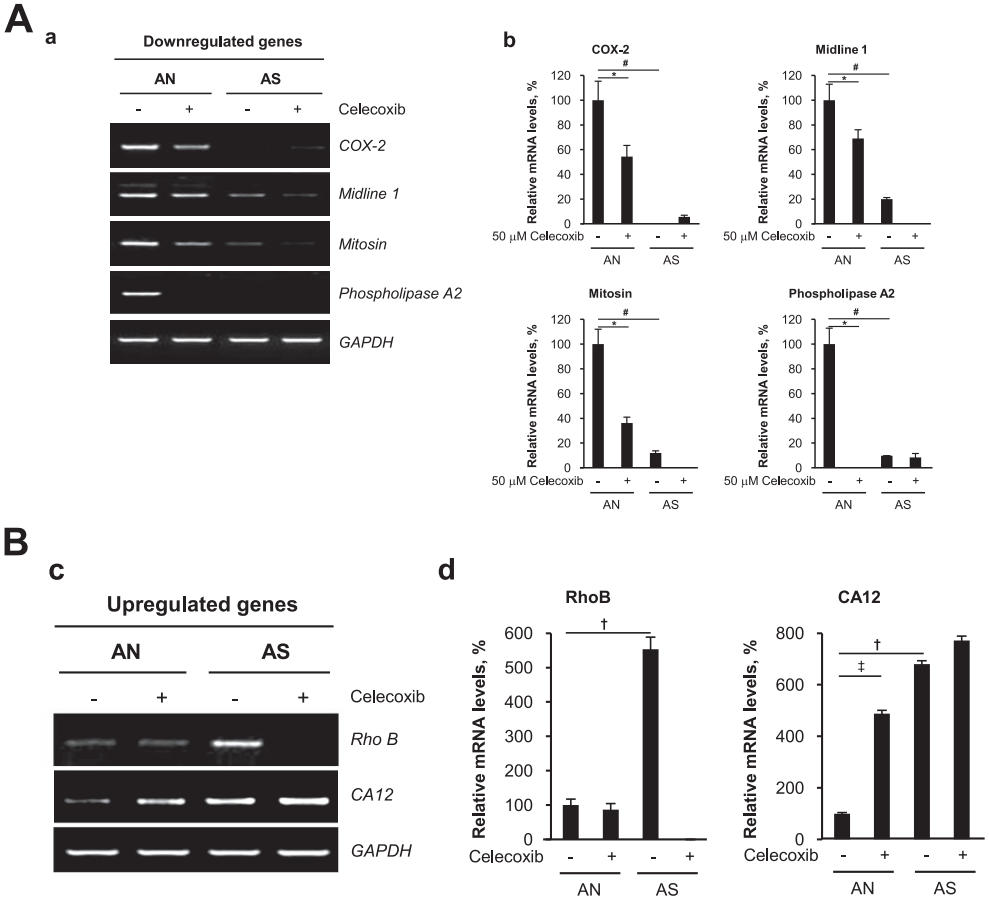

Fig. 4. Confirmation of selected gene data by RT-PCR and Western blots. Synchronously downregulated (midilin1, mitosin, phospholipase A2) (**A**) or upregulated (carbonic anhydrase 12, RhoB) genes (**B**) by COX-2 siRNA and celecoxib were confirmed by RT-PCR. * (celecoxib – *vs* celecoxib + in AN cells) and # (AN cells *vs* AS cells without celecoxib) are $p < 0.05$. -; 0.1% DMSO control, **Celecoxib**; 50 μM Celecoxib. The data are representatives of at least three independent experiments. The levels of each gene were quantified using Multi Gauge V3.0 program.

reported that COX-2 overexpression significantly prolongs IR-induced G_2 arrest, which celecoxib can reverse.[15,27] This suggests that celecoxib could regulate expression of molecules acting on cell cycle and result in radiosensitization. Therefore, we examined whether the protein levels of mitosin (group 1) and RhoB (group 3) change by celecoxib in irradiated cells (Fig. 5).

First, AN and AS cells were treated with celecoxib ± IR and then after 24 h, the cells were harvested. The changes of mitosin and RhoB proteins were monitered using Western blotting. Mitosin protein levels decreased in AS cells compared to AN cells and also decreased following celecoxib treatment in AN cells. In irradiated cells, mitosin downregulation by celecoxib was more prominent in AN cells than in AS cells (Fig. 5A). RhoB protein levels increased in AS cells compared to that in AN cells and increased after celecoxib treatment in AN cells but decreased in AS cells. In irradiated cells, elevated RhoB expression by celecoxib in AN cells was more prominent than in the unirradiated cells, and downregulation of the protein by celecoxib in AS cells was also more prominent than in the unirradiated cells. These results show that mitosin and RhoB are regulated by celecoxib dependent on COX-2 expression in cells. The microarray and western blotting data were consistent and therefore determined to be reliable.

Next, to investigate whether the COX-2 dependent differential regulation of mitosin and RhoB by celecoxib in irradiated cancer cells is general phenomenon, we compared to protein levels of mitosin and RhoB that were changed by celecoxib ± IR in stably COX-2 overexpressing HCT-116 colon cancer cells and control HCT116-Mock cells. In contrast to AN/AS cells, basal expression of mitosin was lower in HCT-116-COX-2 cells compared to Mock cells (Fig. 5B). However, downregulation of mitosin by celecoxib in IR-exposed cells was also dependent on COX-2 expression in the HCT-116-Mock/COX-2 cells as well as AN/AS cell system. We also tried to confirm the change in RhoB expression in HCT116-COX-2 cells but could not detect any western

band and found that HCT-116 colon cancer cells express only very low level of RhoB (Fig. 5B). In summary, mitosin was consistently regulated by celecoxib in a COX-2 expression dependent manner, and therefore determined to be a candidate target molecule for the radiosensitizing effect of celecoxib. In case of RhoB, it may be a candidate molecule but further research is needed with other COX-2-low or -high expressing cell systems.

Identification of genes specifically regulated by celecoxib in irradiated cancer cells

We further analyzed celecoxib-regulated genes under irradiated conditions to investigate the role of celecoxib on cancer cell radiosensitivity. Genes that significantly changed expression (a common set of genes that changed > two-fold and genes changed significantly by ANOVA test) following celecoxib treatment (first group) were compared to genes that changed expression following celecoxib and 6 Gy IR

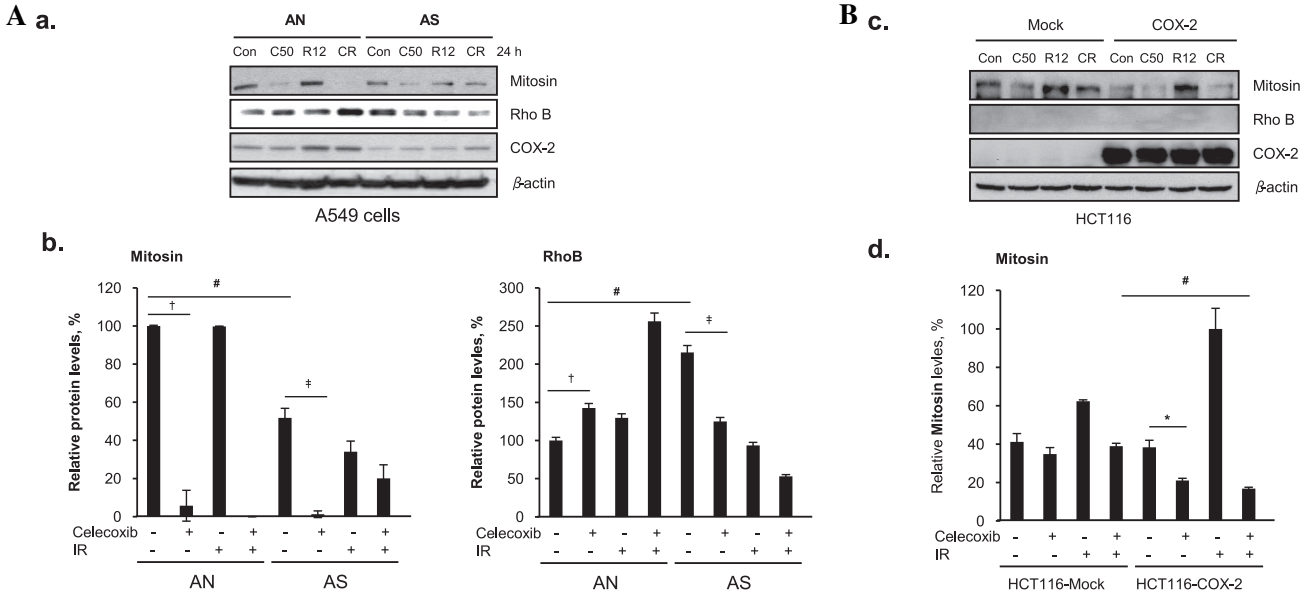

Fig. 5. Mitosin and RhoB may be candidates for radiosensitization by celecoxib in COX-2 overexpressing cell lines. A549-AN/-AS (**A**) and HCT116-Mock/–COX-2 (**B**) cells were treated with 50 μM celecoxib in unirradiated or irradiated condition, and then after 24 h, the cells were harvested. Expression levels of RhoB and mitosin were analyzed by Western blots and were quantified using Multi Gauge V3.0 program . The data are representatives of at least three independent experiments. **Con**; 0.1% DMSO control, **C50**; 50 μM Celecoxib, **R12**; 12 Gy irradiation, **CR**; 50 μM Celecoxib + 12 Gy irradiation. † , #, and ‡ are represented $p < 0.05$. The levels of each gene and protein were quantified using Multi Gauge V3.0 program. All experiments and measurements were done at least in triplicate.

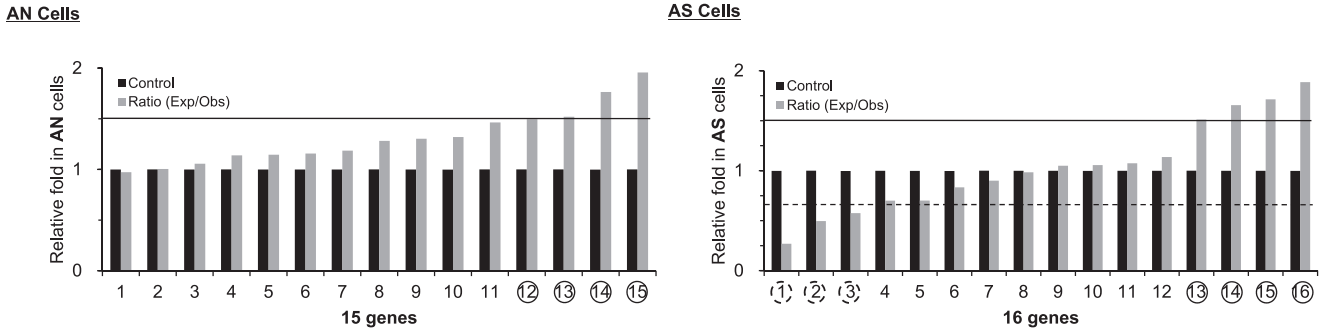

Fig. 6. Specifically regulated genes by celecoxib in irradiated AN or AS cells. AN and AS cells were treated with 6 Gy IR and/or 50 μM celecoxib for 24 h. Genes that significantly changed expression following celecoxib alone treatment (first group) were compared to genes that changed expression following celecoxib and IR treatment (second group). Common sets of these two groups were obtained in AN (15 genes) or AS (16 genes) cells. Then we identified genes that changed by more than 1.5 times the expected additive value, and the ratio of expected and observed values were calculated. The genes with the ratio was below 0.67 (circle with dotted line) or above 1.5 (circle with solid line) were marked. Circled genes mean the genetic change after combined celecoxib and IR treatment is greater than 1.5 times the expected additive change in any (down- or upregulation) direction.

treatment (second group). A common set of these two groups were obtained to find genes that were specifically regulated by celecoxib in irradiated cells. Fifteen and 16 genes were shown to be specifically regulated by celecoxib in irradiated AN and AS cells, respectively (Fig. 6 and Table 4).

Among these genes, we searched ones changed in a more than additive manner by combined celecoxib and IR treatment. Additive change was assumed to be a multiplication

Table 4. Celecoxib-regulated genes in irradiated cells.

	Accession No.	Symbol	Common Name	Control	Celx	6 Gy IR	IR + Celx	Exp*	Obs**	Ratio (Exp/Obs)
AN cells	**(15 genes)**									
1	AA706968	ZWINT	ZW10 interactor	1.00	0.27	0.71	0.20	0.19	0.20	0.97
2	AA454572		zx74g06.s1 Soares ovary tumor NbHOT Homo sapiens cDNA clone IMAGE:809530 3', mRNA sequence.	1.00	0.30	0.64	0.19	0.19	0.19	1.00
3	AA485743	FLJ14675	hypothetical protein FLJ14675	1.00	0.46	1.05	0.46	0.49	0.46	1.06
4	AA454098	KIF23	kinesin family member 23	1.00	0.28	0.76	0.18	0.21	0.18	1.14
5	AI985549	SRP46	Splicing factor, arginine/serine-rich, 46 kD	1.00	0.45	1.18	0.47	0.54	0.47	1.15
6	N74524	TUBB5	tubulin, beta, 5	1.00	0.44	1.00	0.38	0.44	0.38	1.16
7	R16073	INSL4	insulin-like 4 (placenta)	1.00	0.16	1.07	0.14	0.17	0.14	1.19
8	AI984983	PLA2G4A	phospholipase A2, group IVA (cytosolic, calcium-dependent)	1.00	0.31	1.48	0.36	0.46	0.36	1.28
9	H63096	HLA-DMB	major histocompatibility complex, class II, DM beta	1.00	0.30	1.10	0.25	0.33	0.25	1.30
10	H93328	C20orf16	chromosome 20 open reading frame 16	1.00	5.85	1.05	4.66	6.15	4.66	1.32
11	AA418045	RFX5	regulatory factor X, 5 (influences HLA class II expression)	1.00	0.38	1.82	0.48	0.70	0.48	1.46
12	AW073291	AGR2	**anterior gradient 2 homolog (Xenopus laevis)**	1.00	0.37	1.65	0.41	0.61	0.41	**1.50**
13	AW082097	PI3	**protease inhibitor 3, skin-derived (SKALP)**	1.00	0.40	1.67	0.44	0.67	0.44	**1.52**
14	AA115877	SERPINI1	**serine (or cysteine) proteinase inhibitor, clade I (neuroserpin), member 1**	1.00	0.37	2.10	0.44	0.77	0.44	**1.76**
15	AA278384	CDC2	**cell division cycle 2, G1 to S and G2 to M**	1.00	0.21	0.96	0.10	0.20	0.10	**1.96**
AS cells	**(16 genes)**									
1	AA424695		zv33a02.s1 Soares ovary tumor NbHOT Homo sapiens cDNA clone IMAGE:755402 3' similar to gb:M59911 INTEGRIN ALPHA-3 (HUMAN);, mRNA sequence.	1.00	2.67	0.92	9.13	2.45	9.13	**0.27**
2	AA664040	WARS	tryptophanyl-tRNA synthetase	1.00	2.27	0.85	3.88	1.92	3.88	**0.50**
3	AA894927	ASNS	asparagine synthetase	1.00	3.18	0.52	2.90	1.67	2.90	**0.58**
4	H93328	C20orf16	chromosome 20 open reading frame 16	1.00	2.36	1.09	3.68	2.57	3.68	0.70
5	AI361330	MTHFD2	methylene tetrahydrofolate dehydrogenase (NAD + dependent), methenyltetrahydrofolate cyclohydrolase	1.00	2.08	0.57	1.69	1.18	1.69	0.70
6	N74524	TUBB5	tubulin, beta, 5	1.00	0.40	0.70	0.33	0.28	0.33	0.83
7	W95001	CDC25C	cell division cycle 25C	1.00	0.41	0.51	0.24	0.21	0.24	0.90
8	AA458838	PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1	1.00	2.49	1.22	3.10	3.04	3.10	0.98
9	AI335279	C20orf97	chromosome 20 open reading frame 97	1.00	6.04	0.87	5.03	5.28	5.03	1.05
10	AA015892		ze40c09.s1 Soares retina N2b4HR Homo sapiens cDNA clone IMAGE:361456 3' similar to gb:S62138 GROWTH ARREST AND DNA-DAMAGE-INDUCIBLE PROTEIN GADD153 (HUMAN);, mRNA sequence.	1.00	2.54	1.09	2.62	2.77	2.62	1.06
11	W65461	DUSP5	dual specificity phosphatase 5	1.00	3.53	1.09	3.58	3.84	3.58	1.07
12	AW074995	LOC51149	truncated calcium binding protein	1.00	2.45	1.18	2.55	2.90	2.55	1.14
13	W72033	ARHI	**ras homolog gene family, member I**	1.00	0.29	1.31	0.25	0.38	0.25	**1.51**
14	AA598640	MID1	**midline 1 (Opitz/BBB syndrome)**	1.00	0.44	1.52	0.41	0.68	0.41	**1.66**
15	AI826909	APOBEC3B	**apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3B**	1.00	0.49	0.77	0.22	0.37	0.22	**1.71**
16	AI340905	CCND3	**cyclin D3**	1.00	0.45	1.70	0.40	0.76	0.40	**1.89**

Celx; 50 μM celecoxib, IR; 6 Gy γ-irradiation, * Exp; Expected values of IR +Celecoxib are calculated by multiplication of changed values by either celecoxib and IR alone treatment. ** Obs; Observed values of IR +Celecoxib are changed-values by combined treatment of IR and celecoxib. If an Exp/Obs ratio is below 0.67 or over 1.5, we regarded the genes changed in a more than additive manner by combined IR and celecoxib treatment.

of the changes due to each individual treatment. Then we selected genes with levels that had changed by more than 1.5 times the expected additive change to select genes that celecoxib and IR may regulate synergistically. Four genes (anterior gradient 2 homolog, protease inhibitor 3, serine/cystein proteinase inhibitor and cell division cycle 2) and seven genes (trytophanyl-tRNA synthetase, asparagines synthetase, ras homolog gene family, midiline 1, apolipoprotein B mRNA editing enzyme and cyclin D3) were changed by more than 1.5 times the expected additive value in AN and AS cells, respectively (Fig. 6 and Table 4). Several of these genes may be synergistically regulated by celecoxib and IR, and may be additional candidates for further investigation of genes or pathways responsible for celecoxib's radiosensitizing effect. Among these 11 genes, cell cycle-related gene expression (cell division cycle 2, midline 1, and cyclin D3) decreased about 1.7–2 times more than expected in AN or AS cells, indicating that these genes warrant further investigation.

DISCUSSION

In this study, we systematically analyzed differentially expressed genes by COX-2 expression, a COX-2 specific inhibitor (celecoxib), or combined IR and celecoxib treatment. We found that COX-2 knockdown changed various genes related to energy metabolism, angiogenesis, hypoxia-regulation and cell cycle. Recently, John-Aryankalayil M *et al.* reported that COX-2 siRNA rarely altered gene expression in PC3 human prostate carcinoma cells.[8] The discrepancies between these two studies are not clear, but the latter used a prostate cancer cell line that was transiently transfected with COX-2 siRNA, while we used a lung cancer cell line stably expressing COX-2 siRNA. Therefore, our system may provide a more stable COX-2 knockdown and more homogeneous cell population. This may be the underlying reason for the different microarray outcomes. According to our observations, COX-2 seems to regulate genes related to various intracellular processes (Table 1). A number of previous reports suggest that COX-2 is involved in various intracellular functions and signaling pathways, consistent with our findings.[5,7,10]

We also found that celecoxib, a COX-2 specific inhibitor, changed many genes related to signal transduction, enzymes, cell cycle regulation, and nucleic acid binding in both COX-2-overexpressing (AN) and -knockdown (AS) cells. Among these genes, celecoxib changed eight genes common to both AN and AS cells. Four of the eight are related to cell cycle regulation, suggesting that celecoxib may intimately regulate cell cycle in a COX-2-independent manner (Fig. 2). Several reports also pointed out that COX-2 specific or non-specific inhibitors regulate cell cycle-related gene expression.[9,28,29] John-Aryankalayil M *et al.* reported that COX-2 specific inhibitor, NS-398 and COX-2 nonspecific inhibitor, ibuprofen resulted in differential expression of COX-2 dependent and COX-2 independent targets.[8] Sagiv E. *et al.* also reported that celecoxib selectively inhibits the growth of transformed intestinal epithelial cells and induced changes in the protein expression of tumor related genes.[30] These previous studies strongly supported our results that celecoxib regulated expression of genes acting on cell cycle dependent or independent of COX-2.

However, no previous reports have investigated the genetic changes after celecoxib treatment according to changes in COX-2 expression in a single cell line. Therefore, the current study using a COX-2-overexpressing cell line and its COX-2 knockdown cells may provide a further evidence of COX-2-independent regulation of cell cycle-related genes by celecoxib. We also examined the effect of celecoxib in normal and cancer cells, and recently reported that celecoxib showed greater cytotoxic effects in lung cancer cells (A549, NCI-H460, and MOR-P) compared to a normal cell line (bronchial epithelial cell line) regardless of COX-2 expression level.[31] This suggests that lung cancer cells may be more susceptible to celecoxib compared to normal bronchial epithelial cells. Taken together, these results suggest that the understanding of molecular mechanism of multiple targets regulated by celecoxib may give useful clues in clinical approach.

Interestingly, celecoxib downregulated hsp70 gene expression and upregulated the apoptosis-related gene, Par-4, in only AS cells (Supplementary Table 1). Ethridge *et al.* reported that celecoxib downregulated hsp70 expression.[32] Hsp 70 functions as a molecular chaperone, regulating protein folding, stability or activity.[33] Par-4 overexpression was sufficient to induce apoptosis in most cancer cells, but not in normal or immortalized cells.[34] Par-4 regulation by NSAID has also been previously reported.[35] These findings imply that celecoxib may also regulate the chaperone system and may induce apoptosis using par-4-related pathways. Further, these effects may occur in a COX-2-dependent manner, in contrast to cell cycle regulation by celecoxib. Further research is needed and may be useful since these two molecules have been considered as important targets for antineoplastic or radiosensitizing effects.[33,34]

We previously reported that radiosensitization by COX-2 specific inhibitors occurs in a COX-2-dependent manner, whereas the cytotoxic effects of these drugs are COX-2-independent in the same cells.[15,36] In order to identify candidate molecules responsible for the radiosensitization by a COX-2 specific inhibitor, we analyzed the microarray data using two different methods. First, we identified a set of differentially regulated genes common to COX-2 knockdown and celecoxib treatment. These synchronously regulated genes may contain genes changed by celecoxib in a COX-2-dependent manner. We found 27 genes synchronously regulated by COX-2 and celecoxib (Table 2). We selected five genes with known functions (midline 1, mitosin, phospholipase A2, RhoB and CA12) for further analyses. The RT-PCR

and Western blot data for the five genes were similar to the microarray data. Since we previously proposed that the underlying mechanism for radiosensitization by celecoxib may be related to regulating IR-induced G_2/M arrest,[15,37] we focused on two cell cycle-related genes, RhoB and mitosin. Celecoxib changed the expressions of these two proteins in a COX-2-dependent manner in irradiated COX-2-low or overexpressing cancer cells (Fig. 5). Therefore, mitosin and RhoB may have a value for further research to explore the underlying mechanisms of celecoxib's COX-2 dependent radiosensitization.

Second, we selected genes regulated specifically by celecoxib in irradiated cancer cells (Table 4). A subset of these genes that changed following combined celecoxib and IR treatment in a more than additive manner, compared to either treatment alone, was searched. Eleven genes appeared to be regulated by celecoxib and IR treatment in a more than additive manner (Fig. 6 and Table 4). We again focused on the cell cycle-related genes (cell division cycle 2, midline 1, and cyclin D3). These genes may be involved in radiosensitization by celecoxib since radiosensitization by a drug may be mediated by cooperative antineoplastic interactions at the molecular level between the drug and IR in cancer cells. These molecules, identified using two different methods, may provide important clues to define the responsible molecules or pathways for the radiosensitizing effect by celecoxib.

In conclusion, we propose that COX-2 regulates genes related to various intracellular processes in cancer cells including energy metabolism, angiogenesis, hypoxia-regulation and cell cycle regulation. We observed that celecoxib also regulates genes involved in various intracellular functions including signal transduction, enzyme regulation, cell cycle regulation, and nucleic acid binding. Among these, celecoxib seems to change the genes related to cell cycle regulation in a COX-2-independent manner, but chaperone- and apoptosis-related gene regulation may be COX-2-dependent. We also identified genes that may be involved in COX-2-dependent radiosensitization by celecoxib using two different analytical methods with current microarray data. These candidate molecules may be important for further research. Our results may provide useful provisions to further understand the role of COX-2 in cells and to develop an applicable strategy using celecoxib in cancer radiotherapy.

ACKNOWLEGEMENTS

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