Genomic profiling of decreased DNA damage response in human squamous carcinoma cells

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Abstract. A significant problem in the use of radiation and chemotherapy drugs is the development of resistance to these agents by recurrent and metastatic tumors. A number of mechanisms have been proposed for chemotherapy and radiation resistance. Previous studies have suggested that resistant cancer cells are more tolerant of DNA damage than sensitive cells. Overexpression of the multiple drug resistance gene P-glycoprotein, which acts as a drug efflux pump, has been implicated in drug resistance. The glutathione-S-X gene has also been shown to be involved in drug resistance. The increased expression of a number of proto-oncogenes, including AP-1, c-myc and ras, has been associated with chemotherapy resistance. Given the number of reported radiation and chemotherapy resistance genes in cancer, we took a global gene expression approach to examine differences between sensitive and resistant cells and their response to different types of DNA damage. We demonstrated increased numbers of responsive genes in sensitive normal epidermal keratinocytes compared to resistant squamous cell carcinoma cells, regardless of the type of DNA damage. Our results also show new genes that may be responsible for chemotherapy resistance in cancer cells, and differences in how sensitive and resistant cells respond to specific types of DNA damage.

Introduction

In patients with advanced cancer, long term, disease-free and survival rates are low. For example, half the number of patients with advanced head and neck squamous cell carcinoma will experience recurrence in 2 years, with 25% developing metastatic disease (1). Chemotherapy has been integrated with radiation and surgery for locally-advanced disease. The addition of chemotherapy to surgery and radiotherapy improves clinical outcomes in patients with advanced local disease (2-4). Concomitant chemoradiotherapy was associated with absolute survival benefit at 5 years follow-up (5). The combination of cis-diamminedichloroplatinum(II) (cisplatin) and 5-fluorouracil (5-FU) has established activity against squamous cell carcinoma of the head and neck region and has been considered standard therapy for many years (6). Induction chemotherapy with these drugs produces high overall response rates, with complete response in half the number of patients (7). These protocols have defined new treatment paradigms for cancer patients (8).

Anti-proliferative cancer therapy, such as radiation and chemotherapy, is an important part of cancer treatment and is curative for certain types of cancer. These therapies have resulted in improved cure rates when used as adjuvants to surgery. In metastatic disease, anti-proliferative therapy has an increasing role in reducing tumor burden and improving survival. New agents target specific molecules expressed by tumor cells, such as telomerase (9). However, anti-proliferative therapy has significant limitations, among which are toxic effects on normal cells. One of these effects is DNA damage, which induces growth arrest or cell death (reviewed in ref. 10). Understanding how anti-proliferative therapy induces DNA damage and the mechanisms by which normal and cancer cells repair these defects is fundamental to improving therapeutic response, minimizing toxic side effects and maximizing patient survival.

A significant problem in the use of radiation and chemotherapy drugs is the development of resistance to these agents by recurrent and metastatic tumors. A number of mechanisms have been proposed for chemotherapy and radiation resistance. Previous studies have suggested that resistant cancer cells are more tolerant of DNA damage than sensitive cells (11). Overexpression of the multiple drug resistance gene P-glycoprotein, which acts as a drug efflux pump, has been implicated in drug resistance (12). The glutathione-S-X gene has also been shown to be involved in drug resistance (13). Increased expression of a number of proto-oncogenes, including AP-1, c-myc and ras, has been associated with chemotherapy resistance (14-16). Given the number of reported radiation and chemotherapy resistance genes in cancer, we took a global gene expression approach to examine the differences between sensitive and resistant cells and their response to different types of DNA damage. We demonstrated increased numbers of responsive genes in sensitive normal epidermal keratinocytes as compared to resistant squamous cell carcinoma cells, regardless of the type of DNA damage. Our results also show new genes that may be responsible for chemotherapy resistance in cancer cells, and differences in how sensitive and resistant cells respond to specific types of DNA damage.

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Table I. Gene expression changes between NHEK and SCC25 cells (2985 differentially-expressed genes).

Accession	Gene symbol	Gene name	Fold change
BG532690	ITGA4	integrin alpha 4	130.0
NM_004502	HOXB7	homeo box B7	59.2
NM_002427	MMP13	matrix metalloproteinase 13	56.9
AF213459	EPHA3	EPH receptor A3	45.6
NM_005048	PTHR2	parathyroid hormone receptor 2	43.2
AI677888	ORAOV1	oral cancer overexpressed 1	32.5
NM_005375	MYB	v-myb myeloblastosis viral oncogene homolog	32.1
NM_015869	PPARG	PPAR gamma	26.8
NM_000280	PAX6	paired box gene 6 (aniridia, keratitis)	25.3
L37882	FZD2	frizzled homolog 2 (*Drosophila*)	22.8
NM_002876	RAD51C	RAD51 homolog C (*S. cerevisiae*)	18.1
AA524412	TK2	thymidine kinase 2	16.2
BC043596	FANCB	Fanconi anemia, complementation group B	14.7
AF074717	RAD1	RAD1 homolog (*S. pombe*)	14.4
NM_002448	MSX1	msh homeo box homolog 1 (*Drosophila*)	11.9
NM_002006	FGF2	fibroblast growth factor 2 (basic)	9.3
M60316	BMP7	bone morphogenetic protein 7	8.9
NM_022045	MTBP	Mdm2, transformed 3T3 cell double minute 2	8.9
NM_003824	FADD	Fas (TNFRSF6)-associated via death domain	7.8
NM_021138	TRAF2	TNF receptor-associated factor 2	7.8
AA761980	RAD52B	RAD52 homolog B (*S. cerevisiae*)	7.5
AF202640	GPRC5B	G protein-coupled receptor, family C, group 5, B	7.2
AW592604	TOP1MT	topoisomerase (DNA) I, mitochondrial	6.5
NM_001071	TYMS	thymidylate synthetase	6.4
AF034759	MSH5	mutS homolog 5 (*E. coli*)	5.9
NM_000853	GSTT1	glutathione S-transferase theta 1	5.9
AK022829	XRCC3	X-ray repair complementing defective repair 3	5.7
NM_012222	MUTYH	mutY homolog (*E. coli*)	5.6
AW193698	TGFBR3	transforming growth factor, beta receptor III	5.5
AY034104	POLE4	polymerase (DNA-directed), epsilon 4	5.0
NM_001983	ERCC1	excision repair cross-complementing, group 1	-5.0
BE467688	CCND2	cyclin D2	-5.1
AF104013	BMP8B	bone morphogenetic protein 8b	-5.2
AI027678	MTSS1	metastasis suppressor 1	-5.3
NM_003236	TGFA	transforming growth factor, alpha	-5.3
NM_001941	DSC3	desmocollin 3	-6.9
AI703321	WNT5A	wingless-type MMTV integration site family, 5A	-7.6
S74774	FYN	FYN oncogene	-7.9
BC001338	RHOD	ras homolog gene family, member D	-8.9
NM_001942	DSG1	desmoglein 1	-14.3
U38945	CDKN2A	cyclin-dependent kinase inhibitor 2A (p16)	-15.1
BF478120	RECQL5	RecQ protein-like 5	-19.4
AI814274	SBSN	suprabasin	-76.9
NM_006945	SPRR2A	small proline-rich protein 2A	-231.6
NM_002965	S100A9	S100 calcium binding protein A9 (calgranulin B)	-347.0
BC002690	KRT14	keratin 14	-482.8
NM_005547	IVL	involucrin	-566.2
NM_002575	SERPINB2	serine proteinase inhibitor, clade B, member 2	-894.1

Materials and methods

Cell culture. The normal human epidermal keratinocyte strain NHEK was purchased from Clonetics. The human squamous cell carcinoma line SCC25 used in this study was derived from an advanced chemoradioresistant head and neck squamous cell carcinoma purchased from the American Type Culture Collection. SCC25 cells were cultured in Dulbecco's modified

Eagle's medium (DMEM), 10% fetal bovine serum, 40 μg/ml gentamicin at 37˚C in a humidified atmosphere of 5% CO_2. NHEK cells were expanded in keratinocyte growth medium (Clonetics) prior to culture in DMEM, 10% fetal bovine serum, 40 μg/ml gentamicin to minimize variability in culture methods. NHEK and SCC25 cultures were treated with 1 or 6 Gy ionizing radiation from a ^{60}Co source (Norris Comprehensive Cancer Center, University of Southern California, Los Angeles, CA), 2 or 8 μg/ml cis-diamminedichloroplatinum(II), 10 or 30 μg/ml 5-FU, or 10 μM BIBR1532 (telomerase inhibitor, 9) for 16 h. The respective dosages of ionizing radiation, cisplatin and 5-FU were chosen based on their ability to produce a 50% reduction in cell viability 48 h after treatment.

RNA extraction and gene expression profiling. Total-RNA was extracted from cell cultures using a commercially available kit (RNeasy, Qiagen, Valencia, CA). Three independent samples from each group were used in this gene expression analysis. The integrity of ribosomal RNA bands was confirmed by Northern gel electrophoresis. Total-RNA (10 μg) with spike in controls was first reverse transcribed using a T7-Oligo(dT) promoter primer in the first-strand cDNA synthesis reaction. Following RNase H-mediated second-strand cDNA synthesis, the double-stranded cDNA was purified and served as a template in the subsequent *in vitro* transcription (IVT) reaction. The IVT reaction was carried out in the presence of T7 RNA polymerase and a biotinylated nucleotide analog/ribonucleotide mix for complementary RNA (cRNA) amplification and biotin labeling. The biotinylated cRNA targets were then purified, fragmented and hybridized to Affymetrix GeneChip expression arrays (Santa Clara, CA). The human genome U133 microarray was used to interrogate transcripts in each sample (Genomics Core Facility, Children's Hospital, Los Angeles, CA). After washing, hybridization signals were detected using streptavidin conjugated phycoerythrin. Affymetrix GCOS software was used to generate raw gene expression scores and normalized to the relative hybridization signal from each experiment. All gene expression scores were set to a minimum value of 2 x the background, determined by GCOS software, in order to minimize noise associated with less robust measurements of rare transcripts. Normalized gene expression data was imported into dChip software (http://www.biostat.harvard.edu/complab/dchip) for hierarchical clustering analysis using the average linkage algorithm. Raw data was analyzed for quality control and the significance of differential gene expression was determined using the t-test ($p<0.005$) and ratio analysis (>2-fold).

Results

Initially, we compared gene expression between NHEK cells and the cancer cell line SCC25 (Table I). This comparison identified almost 3000 differentially-expressed genes. A number of DNA damage response genes were upregulated in SCC25 cells, including RAD51C (18.1-fold), the Fanconi anemia complementation group B (14.7-fold), RAD1 homolog (14.4-fold), RAD52 homolog B (7.5-fold), the mutS homolog 5 (5.9-fold), X-ray repair complementing defective repair 3 (5.7-fold) and the mutY homolog (5.6-fold). DNA synthesis genes were also upregulated, including mitochondrial topoisomerase (DNA) I (6.5-fold), thymidylate synthetase (6.4-fold) and DNA-directed polymerase ε4 (5.0-fold). The upregulation of viral oncogene homolog v-myb (32.1-fold), Mdm2-transformed 3T3 cell double minute 2 (8.9-fold) and glutathione S-transferase θ1 (5.9-fold) genes was also noted. Downregulation of excision repair cross-complementing group 1 (-5.0-fold) was observed as well. These results indicate that the expression of genes regulating DNA synthesis and damage response is upregulated in SCC25 cells compared to normal keratinocytes.

To determine the response of normal keratinocytes and squamous cell carcinoma cells to different forms of DNA damage, we first treated NHEK or SCC25 cells with ionizing radiation. Expression of 840 genes in NHEK cells was altered by ionizing radiation compared to 468 differentially-expressed genes in the SCC25 line. As shown in Table II, expression of terminal differentiation gene products was higher in irradiated NHEK cells (late cornified envelope 3D, 24.3-fold; filaggrin, 8.9-fold; transglutaminase 5, 8.8-fold). Expression of certain growth factor and receptor mRNAs was upregulated (connective tissue growth factor, 13.3-fold; transforming growth factor ß2, 8.0-fold; fibroblast growth factor 11, 6.4-fold) while others were downregulated (insulin-like growth factor 1 receptor, -5.6-fold; fibroblast growth factor receptor 1, -5.6-fold) in response to ionizing radiation. Ion channel and gap junction gene products were also upregulated by ionizing radiation (sodium channel, nonvoltage gated 1ß, 8.4-fold; gap junction protein α5, 8.3-fold, potassium voltage gated channel 3, 7.1-fold). A number of cell cycle and DNA damage repair mRNAs were downregulated (cell division cycle 2, -5.1-fold; excision repair deficiency group 2, -5.1-fold; Fanconi anemia complementation group A, -5.4-fold; topoisomerase 1, -7.4-fold; aurora kinase B, -7.4-fold; cell division cycle 25C, -12.4-fold; cyclin B2, -13.5-fold; topoisomerase IIα, -18.1-fold; cyclin A2, -24.7-fold; cyclin B1, -31.8-fold). These results indicate that downregulation of cell cycle regulatory gene expression is associated with increased terminal differentiation of NHEK cells in response to ionizing radiation.

In contrast, SCC25 cells did not exhibit the same profile as differentially-expressed genes in response to ionizing radiation, nor was the magnitude of changes in gene expression as large as in NHEK cells (Table III). Heparin-binding EGF-like growth factor was upregulated 10-fold in radiation-exposed SCC25 cells, while insulin-like growth factor 1 receptor was downregulated (-5.5-fold). Epidermal growth factor receptor (-5.8-fold), fibroblast growth factor receptor 2 (-6.4-fold) and transforming growth factor ß receptor III (-8.2-fold) were also downregulated in SCC25 cells. DNA synthesis and repair gene products were differentially regulated in response to ionizing radiation in SCC25 cells (thymidylate synthetase, 6.4-fold; RAD51-like 1, -5.9-fold; RecQ protein-like 5, -6.1-fold; checkpoint suppressor 1, -6.2-fold). Phospholipase C ε1 and phospholipase D1 were downregulated by -5.0 and -5.3-fold, respectively. These results indicate that cell cycle regulatory genes in SCC25 cells were not as affected by ionizing radiation as NHEK cells.

To determine whether different DNA damaging agents regulated different sets of response genes, we exposed NHEK and SCC25 cells to cisplatin. The expression of 886 genes

Table II. Gene expression changes between NHEK control and irradiated cells (840 differentially-expressed genes).

Accession	Gene symbol	Gene name	Fold change
AF268198	ECG2	esophagus cancer-related gene-2	37.3
NM_002774	KLK6	kallikrein 6	33.6
AB048288	LCE3D	late cornified envelope 3D	24.3
NM_001374	DNASE1L2	deoxyribonuclease I-like 2	23.0
D90427	AZGP1	alpha-2-glycoprotein 1, zinc	20.7
NM_021065	HIST1H3D	histone 1, H3d	18.5
AF144103	CXCL14	chemokine (C-X-C motif) ligand 14	17.2
NM_001264	CDSN	corneodesmosin	14.7
M92934	CTGF	connective tissue growth factor	13.3
NM_002963	S100A7	S100 calcium binding protein A7 (psoriasin 1)	12.7
NM_021571	ICEBERG	ICEBERG caspase-1 inhibitor	11.2
AW970948	NAP1L4	nucleosome assembly protein 1-like 4	9.9
AL356504	FLG	filaggrin	8.9
AF119835	KITLG	KIT ligand	8.9
NM_004245	TGM5	transglutaminase 5	8.8
NM_000336	SCNN1B	sodium channel, nonvoltage-gated 1, beta	8.4
AI692880	GJA5	gap junction protein, alpha 5, (connexin 40)	8.3
M19154	TGFB2	transforming growth factor, beta 2	8.0
BC020868	STAT5B	signal transducer activator of transcription 5B	8.0
NM_004438	EPHA4	Eph receptor A4	8.0
AW117498	FOXO1A	forkhead box O1A	7.4
BE501959	RAB7L1	RAB7, member RAS oncogene family-like 1	7.1
AF302494	KCNE3	potassium voltage-gated channel, member 3	7.1
BE221818	MSCP	mitochondrial solute carrier protein	6.9
AA167669	ROCK1	Rho-associated, coiled-coil protein kinase 1	6.8
AA742293	CREBBP	CREB binding protein	6.6
NM_001668	ARNT	aryl hydrocarbon receptor nuclear translocator	6.5
AL528824	FGF11	fibroblast growth factor 11	6.4
AF078803	CAMK2B	calcium dependent protein kinase II beta	6.2
BF970185	DDB2	damage-specific DNA binding protein 2	5.9
AL713641	NCOA1	Nuclear receptor coactivator 1	5.9
AL561296	E2F2	E2F transcription factor 2	-5.1
NM_001786	CDC2	cell division cycle 2, G1 to S and G2 to M	-5.1
AI918117	ERCC2	excision repair deficiency, group 2 (XPD)	-5.1
AI990816	LAMA1	laminin, alpha 1	-5.1
NM_000135	FANCA	Fanconi anemia, complementation group A	-5.4
BE887449	CROP	cisplatin resistance overexpressed protein	-5.4
N50112	IGF1R	insulin-like growth factor 1 receptor	-5.4
M24899	THRA	thyroid hormone receptor, alpha	-5.5
AK024388	FGFR1	fibroblast growth factor receptor 1	-5.6
R37337	NFKB	nuclear factor kappa B 1	-5.8
AF314174	CASP2	caspase 2	-5.9
M31159	IGFBP3	insulin-like growth factor binding protein 3	-6.2
U86214	CASP10	caspase 10	-6.6
NM_004739	MTA2	metastasis associated 1 family, member 2	-7.0
M57731	CXCL2	chemokine (C-X-C motif) ligand 2	-7.1
AI863675	TOP1	topoisomerase (DNA) I	-7.4
AB011446	AURKB	aurora kinase B	-7.4
NM_003158	STK6	serine/threonine kinase 6	-7.8
AI093546	PRKCE	protein kinase C, epsilon	-8.0
NM_004295	TRAF4	TNF receptor-associated factor 4	-11.0
NM_001790	CDC25C	cell division cycle 25C	-12.4
NM_004701	CCNB2	cyclin B2	-13.5
AU159942	TOP2A	topoisomerase (DNA) II alpha 170kDa	-18.1
AI346350	CCNA2	cyclin A2	-24.7
BE407516	CCNB1	cyclin B1	-31.8
NM_005030	PLK1	polo-like kinase 1 (*Drosophila*)	-53.0

Table III. Gene expression changes between SCC25 control and irradiated cells (468 differentially-expressed genes).

Accession	Gene symbol	Gene name	Fold change
NM_001674	ATF3	activating transcription factor 3	18.7
AK023795	ADAMTS1	a disintegrin-like and metalloprotease type 1	15.3
NM_002923	RGS2	regulator of G-protein signalling 2, 24kDa	11.3
AW362945	CTNNB1	catenin (cadherin-associated protein), beta 1	11.2
AU147698	SLC7A1	solute carrier family 7 member 1	11.0
AF069506	RASD1	RAS, dexamethasone-induced 1	10.9
BE674964	RANBP9	RAN binding protein 9	10.4
M60278	HBEGF	heparin-binding EGF-like growth factor	10.0
AI244908	SOCS3	suppressor of cytokine signaling 3	9.8
NM_003811	TNFSF9	tumor necrosis factor superfamily, member 9	8.6
U69563	RND1	Rho family GTPase 1	7.9
AJ012008	DDAH2	dimethylarginine dimethylaminohydrolase 2	7.8
AI263909	RHOB	ras homolog gene family, member B	7.8
AW249678	HES6	hairy and enhancer of split 6 (*Drosophila*)	7.5
BE617348	TAF10	TATA box binding protein associated factor	7.3
U13021	CASP2	caspase 2	6.5
BG281679	TYMS	thymidylate synthetase	6.4
NM_006945	SPRR2	small proline-rich protein 2A	6.2
AW450572	MAPK4K	MAP kinase kinase kinase kinase 4	6.2
NM_006732	FOSB	FBJ osteosarcoma viral oncogene homolog B	6.2
BF062580	dJ222E13.1	kraken-like	5.9
AF348491	CXCR4	chemokine (C-X-C motif) receptor 4	5.9
AV649337	PTPN2	protein tyrosine phosphatase, non-receptor 2	5.8
AL021977	MAFF	v-maf fibrosarcoma oncogene homolog F	5.8
NM_021724	THRA	thyroid hormone receptor, alpha	5.7
AI423466	CLOCK	clock homolog (mouse)	5.5
NM_003858	CCNK	cyclin K	5.3
AA436887	PCTK2	PCTAIRE protein kinase 2	5.3
NM_004443	EPHB3	EPH receptor B3	5.1
NM_004347	CASP5	caspase 5	5.1
NM_006113	VAV3	vav 3 oncogene	-5.0
AB037860	NFIA	nuclear factor I/A	-5.0
AV717336	BCLAF1	BCL2-associated transcription factor 1	-5.0
NM_016341	PLCE1	phospholipase C, epsilon 1	-5.0
AA132961	PLD1	phospholipase D1, phophatidylcholine-specific	-5.3
NM_003545	HIST1H4E	histone 1, H4e	-5.4
H05812	IGF1R	insulin-like growth factor 1 receptor	-5.5
BF438330	WASL	Wiskott-Aldrich syndrome-like	-5.7
U35004	MAPK8	mitogen-activated protein kinase 8	-5.8
AI684746	RASA3	RAS p21 protein activator 3	-5.8
U48722	EGFR	epidermal growth factor receptor	-5.8
Y15571	RAD51L1	RAD51-like 1 (S. cerevisiae)	-5.9
AF155381	ADAM22	a disintegrin and metalloproteinase domain 22	-6.0
AA910945	PPARA	peroxisome proliferative activated receptor	-6.1
AL136869	RECQL4	RecQ protein-like 5	-6.1
U76622	SMAD3	SMAD, mothers against DPP homolog 3	-6.1
AW051527	CHES1	checkpoint suppressor 1	-6.2
M80634	FGFR2	fibroblast growth factor receptor 2	-6.4
NM_005233	EPHA3	EPH receptor A3	-6.8
AI459140	GSTM3	glutathione S-transferase M3	-6.9
NM_006540	NCOA2	nuclear receptor coactivator 2	-7.2
NM_005923	MAP3K5	MAP kinase kinase kinase 5	-7.4
AW193698	TGFBR3	transforming growth factor, beta receptor III	-8.2
AF032887	FOXO3A	forkhead box O3A	-8.2
NM_016152	RARB	retinoic acid receptor, beta	-8.2
NM_018328	MBD5	methyl-CpG binding domain protein 5	-9.0

Table IV. Gene expression changes between NHEK control and cisplatin-treated cells (886 differentially-expressed genes).

Accession	Gene symbol	Gene name	Fold change
M92934	CTGF	connective tissue growth factor	54.1
NM_002774	KLK6	kallikrein 6	48.7
AF268198	ECG2	esophagus cancer-related gene-2	46.9
X07695	KRT4	keratin 4	26.4
NM_001264	CDSN	corneodesmosin	26.3
BC001131	HIST1H2BG	histone 1, H2bg	24.7
AF144103	CXCL14	chemokine (C-X-C motif) ligand 14	19.6
NM_021571	ICEBERG	ICEBERG caspase-1 inhibitor	16.3
AL356504	FLG	filaggrin	14.6
BC002646	JUN	v-jun sarcoma virus 17 oncogene homolog	14.1
NM_001674	ATF3	activating transcription factor 3	13.5
NM_014058	TMPRSS11E	transmembrane protease, serine 11E	10.8
AB028021	FOXA2	forkhead box A2	9.6
NM_002963	S100A7	S100 calcium binding protein A7 (psoriasin 1)	8.3
NM_004245	TGM5	transglutaminase 5	8.2
AI056872	FOXO3A	forkhead box O3A	7.3
AF348491	CXCR4	chemokine (C-X-C motif) receptor 4	7.0
U40053	CYP51A1	cytochrome P450, family 51, subfamily A	6.8
NM_003914	CCNA1	cyclin A1	6.2
AI914925	STAT3	signal transducer/activator of transcription 3	5.8
U20498	CDKN2D	cyclin-dependent kinase inhibitor 2D (p19)	5.8
AI814274	SBSN	suprabasin	5.4
AI829910	CAMK2D	calcium/calmodulin-dependent protein kinase II	5.3
NM_006846	SPINK5	serine protease inhibitor, Kazal type 5	5.3
NM_002653	PITX1	paired-like homeodomain transcription factor 1	5.2
AA058828	FLT1	Fms-related tyrosine kinase 1	5.0
AL713641	NCOA1	nuclear receptor coactivator 1	5.0
AF035594	PRKCA	protein kinase C, alpha	-5.0
AL031668	EIF2S2	eukaryotic translation initiation factor 2	-5.2
AB011446	AURKB	aurora kinase B	-5.3
NM_004530	MMP2	matrix metalloproteinase 2	-5.3
NM_003593	FOXN1	forkhead box N1	-5.4
AI918117	ERCC2	excision repair cross-complementing 2	-5.5
BG426657	PPARA	peroxisome proliferative activated receptor	-5.8
AL575177	NOG	noggin	-5.8
NM_001237	CCNA2	cyclin A2	-6.3
NM_000142	FGFR3	fibroblast growth factor receptor 3	-6.3
AB017445	XRCC4	X-ray repair complementing 4	-6.3
NM_004701	CCNB2	cyclin B2	-6.4
NM_004994	MMP9	matrix metalloproteinase 9	-6.5
AB030078	FGFR2	fibroblast growth factor receptor 2	-6.6
AW007532	IGFBP5	insulin-like growth factor binding protein 5	-6.9
AU159942	TOP2A	topoisomerase (DNA) II alpha 170 kDa	-7.4
BE407516	CCNB1	cyclin B1	-7.8
NM_019884	GSK3A	glycogen synthase kinase 3 alpha	-11.2
NM_005030	PLK1	polo-like kinase 1 (*Drosophila*)	-11.7

in NHEK cells was altered by cisplatin, compared to 351 differentially-expressed genes in the SCC25 line (Tables IV and V). Expression of terminal differentiation genes was again upregulated in NHEK cells (filaggrin, 14.6-fold; transglutaminase 5, 8.2-fold; suprabasin, 5.4-fold). Specific growth factor receptors were also downregulated (fibroblast growth factor receptor 3, -6.3-fold; fibroblast growth factor receptor 2, -6.6-fold). Cell cycle regulatory proteins were again downregulated by cisplatin in NHEK cells (cyclin A2, -6.3-fold; cyclin B2, -6.4-fold; cyclin B1, -7.8-fold), as were DNA

Table V. Gene expression changes between SCC25 control and cisplatin-treated cells (351 differentially-expressed genes).

Accession	Gene symbol	Gene name	Fold change
NM_001674	ATF3	activating transcription factor 3	28.1
AI343467	INHBA	inhibin, beta A (activin A)	21.1
NM_000963	PTGS2	prostaglandin-endoperoxide synthase 2	15.5
NM_001085	SERPINA3	serine proteinase inhibitor, clade A, member 3	12.7
AW362945	CTNNB1	catenin (cadherin-associated protein), beta 1	8.7
NM_005658	TRAF1	TNF receptor-associated factor 1	7.9
NM_006290	TNFAIP3	tumor necrosis factor, alpha-induced protein 3	7.9
AU147698	SLC7A1	solute carrier family 7 member 1	7.7
AI244908	SOCS3	suppressor of cytokine signaling 3	7.4
BC004257	RET	ret proto-oncogene	7.2
NM_004513	IL16	interleukin 16	7.2
AW249678	HES6	hairy and enhancer of split 6	7.1
NM_001924	GADD45A	growth arrest and DNA-damage-inducible, alpha	7.1
BF062580	dJ222E13.1	kraken-like	6.8
AL031447	THAP3	THAP domain apoptosis associated protein 3	6.8
NM_004591	CCL20	chemokine (C-C motif) ligand 20	6.6
NM_003548	HIST2H4	histone 2, H4	6.6
NM_004443	EPHB3	EPH receptor B3	6.5
U69563	RND	Rho family GTPase 1	6.2
AL049709	GGTL3	gamma-glutamyltransferase-like 3	6.1
AA533080	JARID2	jumonji, AT rich interactive domain 2	6.0
NM_000609	CXCL12	chemokine (C-X-C motif) ligand 12	5.9
AF232905	C1QTNF1	C1q and tumor necrosis factor related protein 1	5.8
NM_000499	CYP1A1	cytochrome P450, family 1, subfamily A1	5.8
AA496799	BCAR3	breast cancer anti-estrogen resistance 3	5.8
NM_000584	IL8	interleukin 8	5.8
NM_003858	CCNK	cyclin K	5.6
M76453	CSF1	colony stimulating factor 1	5.2
AW444761	CDKN2B	cyclin-dependent kinase inhibitor 2B (p15)	5.2
U13223	FOXD4	forkhead box D4	5.1
AW192876	CSNK1E	casein kinase 1, epsilon	5.0
NM_006732	FOSB	FBJ osteosarcoma viral oncogene homolog B	5.0
NM_020525	IL22	interleukin 22	-5.1
AI760295	JAG1	jagged 1	-5.7
NM_017781	CYP2W1	cytochrome P450, family 2, subfamily W, 1	-5.9
AF314174	CASP2	caspase 2	-6.9
NM_016087	WNT16	wingless-type MMTV integration, member 16	-7.2
NM_003521	HIST1H2BM	histone 1, H2bm	-11.7
NM_005030	PLK1	polo-like kinase 1 (*Drosophila*)	-12.5

repair and replication genes (aurora kinase B, -5.3-fold; excision repair cross complementing 2, -5.5-fold; X-ray repair complementing 4, -6.3-fold; topoisomerase IIα, -7.4-fold). These results indicate that a common response to DNA damage exists in NHEK cells.

Similar genes were regulated in SCC25 cells exposed to cisplatin as in those treated with ionizing radiation. This set of genes was markedly different than those observed in NHEK cells. However, certain genes (e.g., polo-like kinase 1) were found downregulated by cisplatin in NHEK and SCC25 cells. Activating transcription factor 3, catenin ß1, suppressor of cytokine signaling 3, Rho family GTPase 1 and cyclin K were upregulated in response to both radiation and cisplatin. Fewer genes were downregulated in SCC25 cells treated with cisplatin than in those treated with ionizing radiation. These results indicate that a common response to DNA damage may exist in SCC25 cells.

We also treated NHEK and SCC25 cells with 5-FU, an inhibitor of DNA synthesis. The expression of 733 genes in NHEK cells was altered by 5-FU, compared to 565 differentially-expressed genes in the SCC25 line. Once again, a similar set of genes was differentially regulated in NHEK cells (Table VI). Terminal differentiation gene products were upregulated (late cornified envelope 3D, 37.2-fold; small proline rich protein 2G, 14.4-fold; filaggrin, 13.0-fold; suprabasin, 6.1-fold). Cell cycle regulatory gene products were downregulated (cell division cycle 2, -5.0-fold; aurora kinase B, -6.5-fold; cyclin B2, -6.5-fold; topoisomerase IIα, -8.7-fold;

Table VI. Gene expression changes between NHEK control and fluorouracil-treated cells (733 differentially-expressed genes).

Accession	Gene symbol	Gene name	Fold change
AF268198	ECG2	esophagus cancer-related gene-2	47.8
D90427	AZGP1	alpha-2-glycoprotein 1, zinc	47.1
AB048288	LCE3D	late cornified envelope 3D	37.2
X07695	KRT4	keratin 4	32.6
NM_001374	DNASE1L2	deoxyribonuclease I-like 2	32.0
AF144103	CXCL14	chemokine (C-X-C motif) ligand 14	30.6
M92934	CTGF	connective tissue growth factor	26.0
BC001131	HIST1H2BG	histone 1, H2bg	22.5
NM_021571	ICEBERG	ICEBERG caspase-1 inhibitor	21.8
AA456642	SPRR2G	small proline-rich protein 2G	14.4
AL356504	FLG	filaggrin	13.0
AI684894	NFKBIB	nuclear factor of kappa B inhibitor, beta	10.3
NM_003914	CCNA1	cyclin A1	9.1
U40053	CYP51A1	cytochrome P450, family 51, subfamily A, 1	7.8
AY185496	SERPINA9	serine proteinase inhibitor, clade A member 9	7.1
BC020868	STAT5B	signal transducer activator of transcription 5B	6.8
AB030077	FGFR2	fibroblast growth factor receptor 2	6.6
AU144916	POLR2B	polymerase (RNA) II polypeptide B	6.5
AW117498	FOXO1A	forkhead box O1A	6.4
AI814274	SBSN	suprabasin	6.1
AA704163	EIF4G3	EIF 4 gamma, 3	6.0
AA742293	CREBBP	CREB binding protein	5.1
BC005842	SUV420H2	suppressor of variegation 4-20 homolog 2	5.0
BC002427	CASP2	caspase 2	-5.0
AF035594	PRKCA	protein kinase C, alpha	-5.0
D88357	CDC2	cell division cycle 2, G1 to S and G2 to M	-5.0
BF340228	IGFBP3	insulin-like growth factor binding protein 3	-5.0
NM_000142	FGFR3	fibroblast growth factor receptor 3	-5.0
NM_004994	MMP9	matrix metalloproteinase 9	-5.1
AF104013	BMP8B	bone morphogenetic protein 8b	-5.2
NM_017789	SEMA4C	semaphorin 4C	-6.5
AB011446	AURKB	aurora kinase B	-6.5
NM_004701	CCNB2	cyclin B2	-6.5
AF043337	IL8	interleukin 8	-7.0
NM_002985	CCL5	chemokine (C-C motif) ligand 5	-7.3
NM_021724	THRA	thyroid hormone receptor alpha	-7.5
BF434653	MAPK1	mitogen-activated protein kinase 1	-7.5
S81491	STAT2	signal transducer and activator of transcription 2	-8.7
AU159942	TOP2	topoisomerase (DNA) II alpha	-8.7
NM_003158	STK6	serine/threonine kinase 6	-9.0
AK026546	CXCL5	chemokine (C-X-C motif) ligand 5	-13.9
NM_005030	PLK1	polo-like kinase 1	-25.0
BE407516	CCNB1	cyclin B1	-28.8

polo-like kinase 1, -25.0-fold, cyclin B1, -28.8-fold). In contrast, SCC25 cells exposed to 5-FU upregulated thymidylate synthetase 13.0-fold, checkpoint suppressor 1 (9.1-fold), catenin ß1 (9.0-fold), RAD52 homolog (5.8-fold) and epidermal growth factor receptor (5.6-fold) genes (Table VII). RecQ protein-like 5 (-6.6-fold) and cyclin B1 (-7.6-fold) were downregulated by 5-FU in SCC25 cells. These results once again indicate a common set of differentially-expressed genes in response to DNA damage specific to NHEK or SCC25 cells.

To compare traditional DNA damaging agents to more targeted drugs, we treated NHEK and SCC25 cells with the telomerase inhibitor BIBR1532. Both early passage NHEK and SCC25 cells express telomerase, which maintains the ends

Table VII. Gene expression changes between SCC25 control and fluorouracil-treated cells (565 differentially-expressed genes).

Accession	Gene symbol	Gene name	Fold change
BG281679	TYMS	thymidylate synthetase	13.0
U12707	WAS	Wiskott-Aldrich syndrome	11.7
AI652000	CENTG3	centaurin, gamma 3	11.1
AI743607	EXT1	exostoses (multiple) 1	10.9
AA496799	BCAR3	breast cancer anti-estrogen resistance 3	10.2
R98192	MARK3	MAP/microtubule affinity-regulating kinase 3	9.1
AA860806	CHES1	checkpoint suppressor 1	9.1
AU147698	SLC7A1	solute carrier family 7 member 1	9.1
AW362945	CTNNB1	catenin, beta 1	9.0
AL049709	GGTL3	gamma-glutamyltransferase-like 3	8.7
AB007970	RASAL2	RAS protein activator-like 2	8.6
AK021554	THRAP2	thyroid hormone receptor associated protein 2	8.4
NM_007272	CTRC	chymotrypsin C (caldecrin)	8.3
NM_017977	AIM1L	absent in melanoma 1-like	8.3
AK024129	CTBP2	C-terminal binding protein 2	8.0
N39535	SAE1	SUMO-1 activating enzyme subunit 1	7.9
BC004257	RET	ret proto-oncogene	7.9
AW959449	CRSP3	cofactor required for Sp1 activation	7.8
AA436887	PCTK2	PCTAIRE protein kinase 2	7.5
AK026286	FOXO3A	forkhead box O3A	7.5
AA284757	CAMK2G	calcium dependent protein kinase II gamma	7.4
AW974998	ARHGAP1	Rho GTPase activating protein 10	7.1
NM_000316	PTHR1	parathyroid hormone receptor 1	7.0
AA618295	IGF1R	insulin-like growth factor 1 receptor	7.0
BU689085	BBX	bobby sox homolog (*Drosophila*)	7.0
NM_002377	MAS1	MAS1 oncogene	6.8
NM_006225	PLCD1	phospholipase C, delta 1	6.5
AU147253	PRKCH	protein kinase C, eta	6.5
AW192876	CSNK1E	casein kinase 1, epsilon	6.1
BC033945	PPP3R1	protein phosphatase 3 (calcineurin B, type I)	5.9
AI401627	TNKS	tankyrase	5.9
NM_002188	IL13	interleukin 13	5.8
AF125950	RAD52	RAD52 homolog (S. cerevisiae)	5.8
BE222450	DATF1	death associated transcription factor 1	5.7
AU156822	EGFR	epidermal growth factor receptor	5.6
BC005055	FOXP1	forkhead box P1	5.5
BF062580	dJ222E13.1	kraken-like	5.5
AI935720	PIB5PA	PI bisphosphate phosphatase, A	5.2
NM_004579	MAP4K2	MAP kinase kinase kinase kinase 2	5.1
AF348491	CXCR4	chemokine (C-X-C motif) receptor 4	5.0
NM_005883	APC2	adenomatosis polyposis coli 2	-5.0
BG054960	TIMP4	tissue inhibitor of metalloproteinase 4	-5.2
NM_005321	HIST1H1E	histone 1, H1e	-5.2
N36160	CROP	cisplatin resistance overexpressed protein	-5.4
AI005407	FOXL1	forkhead box L1	-5.9
AI709406	MARCKS	myristoylated alanine-rich PKC substrate	-6.0
AI765747	SMAD2	SMAD, mothers against DPP homolog 2	-6.2
AL136869	RECQL5	RecQ protein-like 5	-6.6
AF314174	CASP2	caspase 2	-6.9
AU151151	LEPR	leptin receptor	-7.4
N90191	CCNB1	cyclin B1	-7.6
NM_004612	TGFBR1	transforming growth factor, beta receptor I	-8.1

Table VIII. Gene expression changes between NHEK control and BIBR1532-treated cells (499 differentially-expressed genes).

Accession	Gene symbol	Gene name	Fold change
X07695	KRT4	keratin 4	78.8
NM_002888	RARRES1	retinoic acid receptor responder (tazarotene induced) 1	64.4
X02189	ADA	adenosine deaminase	39.3
AF144103	CXCL14	chemokine (C-X-C motif) ligand 14	25.0
BG532690	ITGA4	integrin, alpha 4	21.8
NM_000782	CYP24A1	cytochrome P450, family 24, subfamily A, polypeptide 1	14.7
NM_000930	PLAT1	plasminogen activator, tissue	13.9
AW117498	FOXO1A	forkhead box O1A (rhabdomyosarcoma)	13.5
AF119835	KITLG	KIT ligand	13.0
AA524412	TK2	thymidine kinase 2, mitochondrial	9.8
M92934	CTGF	connective tissue growth factor	9.7
NM_004878	PTGES	prostaglandin E synthase	9.5
AL050262	TLR1	toll-like receptor 1	9.0
NM_002876	RAD51C	RAD51 homolog C (S. cerevisiae)	8.3
AF012536	TNFRSF10C	tumor necrosis factor receptor superfamily, member 10c	8.2
BC020765	SERPINE1	serine (or cysteine) proteinase inhibitor, clade E, member 1	7.9
NM_004887	CXCL14	chemokine (C-X-C motif) ligand 14	7.3
NM_005375	MYB	v-myb myeloblastosis viral oncogene homolog (avian)	7.2
AI758962	EPHA4	EPH receptor A4	7.1
BE219979	IL20RA	interleukin 20 receptor, alpha	6.9
AF118886	VAV3	vav 3 oncogene	6.8
BC005008	CEACAM6	carcinoembryonic antigen-related cell adhesion molecule 6	6.6
BC004877	UNG2	uracil-DNA glycosylase 2	6.1
NM_015400	SMAD3	SMAD, mothers against DPP homolog 3 (*Drosophila*)	6.0
AA742293	CREBBP	CREB binding protein (Rubinstein-Taybi syndrome)	5.9
AF308602	NOTCH1	notch homolog 1, translocation-associated (*Drosophila*)	5.9
BF971923	MAP3K3	mitogen-activated protein kinase kinase kinase 3	5.3
AF072872	FZD1	frizzled homolog 1 (*Drosophila*)	5.3
AY009400	WNT10A	wingless-type MMTV integration site family, member 10A	5.1
U57001	EFNB3	ephrin b3	-5.2
NM_021724	THRA	thyroid hormone receptor, alpha	-5.4
NM_003593	FOXN1	forkhead box N1	-5.4
NM_004994	MMP9	matrix metalloproteinase 9	-5.5
BF196457	DSC2	desmocollin 2	-5.7
AL575177	NOG	noggin	-5.8
AK021881	HIF3A	hypoxia inducible factor 3, alpha subunit	-5.8
NM_002923	RGS2	regulator of G-protein signalling 2, 24kDa	-5.8
U04897	RORA	RAR-related orphan receptor A	-5.8
AK026546	CXCL5	chemokine (C-X-C motif) ligand 5	-5.9
AJ276395	FN1	fibronectin 1	-6.0
BC032003	SPINK6	serine protease inhibitor, Kazal type 6	-6.3
NM_002425	MMP10	matrix metalloproteinase 10 (stromelysin 2)	-6.5
AW007532	IGFBP5	insulin-like growth factor binding protein 5	-6.9
BF110534	RASGEF1B	RasGEF domain family, member 1B	-7.1
H23551	PAK3	P21 (CDKN1A)-activated kinase 3	-7.1
N71063	ADAMTS6	a disintegrin-like and metalloprotease, thrombospondin motif, 6	-8.3
U64094	IL1R2	interleukin 1 receptor, type II	-8.5
NM_000359	TGM1	transglutaminase 1	-8.8
BE671224	STK11	serine/threonine kinase 11 (Peutz-Jeghers syndrome)	-8.9
AF277897	EGFR	epidermal growth factor receptor	-10.6
AV682252	GLIPR1	GLI pathogenesis-related 1 (glioma)	-12.2
AB049591	CNFN	cornifelin	-21.2
NM_000640	IL13RA2	interleukin 13 receptor, alpha 2	-43.5

Table IX. Gene expression changes between SCC25 control and BIBR1532-treated cells (140 differentially-expressed genes).

Accession	Gene symbol	Gene name	Fold change
BC002710	KLK10	kallikrein 10	18.0
NM_005052	RAC3	ras-related C3 botulinum toxin substrate 3	8.8
AU149305	MMP14	matrix metalloproteinase 14 (membrane-inserted)	8.6
AF082185	TRAF4	TNF receptor-associated factor 4	8.2
NM_024302	MMP28	matrix metalloproteinase 28	7.5
NM_000499	CYP1A1	cytochrome P450, family 1, subfamily A, polypeptide 1	6.9
AI800895	MAP4K3	mitogen-activated protein kinase kinase kinase kinase 3	6.4
NM_001552	IGFBP4	insulin-like growth factor binding protein 4	6.1
NM_002428	MMP15	matrix metalloproteinase 15 (membrane-inserted)	6.1
BE965869	RAB40C	RAB40C, member RAS oncogene family	6.0
NM_002899	RBP1	retinol binding protein 1, cellular	5.5
AW192876	CSNK1E	casein kinase 1, epsilon	5.4
AA496799	BCAR3	breast cancer anti-estrogen resistance 3	5.4
AK098058	MAPK12	mitogen-activated protein kinase 12	5.3
AW117498	FOXO1A	forkhead box O1A (rhabdomyosarcoma)	5.3
NM_021114	SPINK2	serine protease inhibitor, Kazal type 2 (acrosin-trypsin inhibitor)	5.0
BG281679	TYMS	thymidylate synthetase	5.0
NM_017781	CYP2W1	cytochrome P450, family 2, subfamily W, polypeptide 1	-5.1
NM_139057	ADAMTS17	a disintegrin and metalloprotease, thrombospondin motif, 17	-5.1
AW341182	DLL3	delta-like 3 (*Drosophila*)	-5.2
BE671224	STK11	serine/threonine kinase 11 (Peutz-Jeghers syndrome)	-5.3
BE311922	CDC42BPB	CDC42 binding protein kinase beta (DMPK-like)	-5.4
BC004490	FOS	v-fos FBJ murine osteosarcoma viral oncogene homolog	-5.5
BF476613	MUC	mucin	-5.6
AF095784	GPR51	G protein-coupled receptor 51	-5.6
AI912696	MAGEE1	melanoma antigen family E, 1	-5.8
AF085825	POLA	polymerase (DNA directed), alpha	-5.9
NM_004573	PLCB2	phospholipase C, beta 2	-5.9
AA016035	TUBGCP2	tubulin, gamma complex associated protein 2	-6.0
U47924	CD4	CD4 antigen	-6.3
N25325	CALM1	calmodulin 1 (phosphorylase kinase, delta)	-6.5
AI459194	EGR1	early growth response 1	-6.5
BC002646	JUN	v-jun sarcoma virus 17 oncogene homolog (avian)	-7.9

of chromosomes. The expression of 499 genes in NHEK cells was altered by BIBR1532, compared to 140 differentially-expressed genes in the SCC25 line (Tables VIII and IX). Treatment with BIBR1532 regulated different sets of genes in both NHEK and SCC25 cells. In NHEK cells, upregulated genes included thymidine kinase 2 (9.8-fold), connective tissue growth factor (9.7-fold), RAD51 homolog C (8.3-fold), vav3 oncogene (6.8-fold), SMAD3 (6.0-fold), CBP (5.9-fold), frizzled homolog 1 (5.3-fold) and Wnt10a (5.1-fold). Downregulated genes included thyroid hormone receptor α (-5-4-fold), desmocollin 2 (-5.7-fold), transglutaminase 1 (-8.8-fold), epidermal growth factor receptor (-10.6-fold) and cornifelin (-21.2-fold). Terminal differentiation gene products that were upregulated by radiation, cisplatin and 5-FU were downregulated by BIBR1532. In SCC25 cells, upregulated genes included Rac3 (8.8-fold), cytochrome P450 family 1 (6.9-fold), casein kinase 1ε (5.4-fold), breast cancer anti-estrogen resistance 3 (5.4-fold) and thymidylate synthetase (5.0-fold). Downregulated genes included δ-like 3 (-5.2-fold), serine/threonine kinase 11 (-5.3-fold), c-fos (-5.5-fold), DNA polymerase α (-5.9-fold), phospholipase C ß2 (-5.9-fold) and c-jun (-7.9-fold). Few classic cell cycle regulatory proteins were affected by BIBR1532 treatment in NHEK and SCC25 cells. These results indicate that the telomerase inhibitor BIBR1532 does not regulate the same gene sets as do DNA damaging agents.

Discussion

The results of this study indicate that squamous carcinoma cells express higher levels of a number of genes involved in DNA replication and repair. These genes include RAD51C, Fanconi anemia complementation group B, RAD1 homolog, RAD52 homolog B, mutS homolog 5, X-ray repair comple-

menting defective repair 3 and the mutY homolog. Other upregulated DNA synthesis genes included mitochondrial topoisomerase (DNA) I, thymidylate synthetase and DNA directed polymerase ε4. Functional studies will be required to determine whether these genes impart, to cancer cells, increased resistance to DNA damage. Additionally, the number of responsive genes was consistently lower in SCC25 cells exposed to radiation, cisplatin, 5-FU and BIBR1532. These data suggest that SCC25 cells exhibit decreased DNA damage response compared to NHEK cells. In order to overcome clinical drug resistance, it will be important to determine which genes contribute to this phenotype.

The response of NHEK cells to DNA damaging agents demonstrates a number of common features. DNA damage induces a number of terminal differentiation genes, including late cornified envelope 3D, corneodesmosin, filaggrin, transglutaminase 5 and suprabasin. It has been shown that ionizing radiation induces filaggrin expression in mouse epidermis (17,18). A number of histone genes were upregulated by DNA damaging agents, which may act to repress transcription or function in DNA repair. Cisplatin crosslinks have been shown to locally override the predefined rotational setting of positioned nucleosomes (19). Activating transcription factor 3 was upregulated by DNA damaging agents in NHEK and SCC25 cells; although this had been demonstrated previously, the function of this gene in this response is unknown (20). Transforming growth factor ß genes were upregulated, which inhibits proliferation of stratified epithelia. This function is consistent with growth inhibition following DNA damage.

Specific classes of genes were downregulated following DNA damage. These groups of genes were inhibited regardless of the type of DNA damage. Both G1 and G2 phase cyclins were inhibited by DNA damaging agents. The G2 phase cyclin-dependent kinase cdc2 was also inhibited, consistent with activation of the G2 checkpoint. DNA replication and repair genes were also downregulated, including FANCA, ERCC2, XPD and topoisomerases. XPD overexpression resulted in cisplatin resistance in glioma cell lines (21). Polo-like kinase 1, which was dramatically downregulated by DNA damage, has been shown to regulate exit from the G2 checkpoint (22). Aurora kinase has been shown to regulate cisplatin resistance in ovarian cancer cells (23). Expression of the forkhead transcription factor FOXO3A is induced by radiation exposure (24), in agreement with our results.

Cells exposed to the telomerase inhibitor BIBR1532 experience loss of telomeric DNA over time, triggering a DNA damage response. Our data indicate that this DNA damage response is substantially different than that induced by direct acting agents. BIBR1532 does not induce differentiation markers in NHEK or SCC25 cells, as do the direct acting agents. The telomerase inhibitor does not inhibit cell cycle regulatory genes in the same manner as radiation, cisplatin and 5-FU. It should be noted that this study evaluated short term response to the telomerase inhibitor, which may be different than that of cells exposed to the drug for weeks or months. However, the SCC25 line was resistant to BIBR1532 even at 400 population doublings, suggesting that generalized drug resistance is a prominent feature of these cells. Future studies will determine how this common pathway of drug resistance can be modulated in cancer cells.

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