Supplementary Data

Supplementary Materials and Methods

Reagents

Antibodies were obtained from the following sources: rabbit anti-epidermal growth factor receptor (EGFR), Alexa® 488 conjugated rabbit anti-EGFR, rabbit anti-pAkt, rabbit anti-Akt, anti-pMEK1/2, rabbit anti-pErk1/2, rabbit anti-γH2AX, anti-rabbit IgG (H+L), F(ab′)2 Fragment (Alexa Fluor 488 Conjugate), mouse anti-phosphotyrosine antibody (p-Tyr-100), and rabbit anti-β actin from Cell Signaling; rabbit anti-EGFR, rabbit anti-pEGFR, and rabbit anti-mouse IgG HRP from Santa Cruz Biotechnology; and goat anti-rabbit IgG HRP from Jackson Laboratories. Prx1 and 2, anti-peroxiredoxin-$SO_{2/3}$, superoxide dismutase (SOD), catalase, and GAPDH antibodies were from Abcam. DMEM/F12 and fetal bovine serum were purchased from Gibco (Invitrogen). Phosphate-buffered saline (PBS) was purchased from Lonza. Erlotinib, Cell Proliferation Kit I (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide [MTT]) was purchased from Roche. Cloned AMV cDNA kit, AlexaFluor594 conjugated cholera toxin subunit B (CT-B), AlexaFluo594 conjugated streptavidin, Topro-3-iodide, and Hoechst nucleic acid stains were purchased from Invitrogen. RNeasy Plus Micro kit (with genomic DNA removal columns) and RNA were later purchased from Qiagen. Bicinchoninic acid (BCA) protein estimation kit and Western Lightning Plus ECL were purchased from Thermo scientific. Glutathione Assay Kit and Lactate Assay Kit II were purchased from BioVision. Methyl-β-cyclodextrin (MβCD) and Lovastatin were purchased from Sigma.

Clonogenic cell survival assay

Radiation-resistant rSCC-61 and the parental SCC-61 cells were trypsinized, resuspended in complete medium, and plated into the six-well culture dishes at a low density. After 4 h of incubation at 37°C, each 6-well plate was subjected to radiation with different doses (0, 1, 2, 4, and 6 Gy) and allowed to incubate for further 10 days for the colony formation. Once formed, the colonies were fixed in methanol and acetic acid (1:1) solution and stained with 0.5% crystal violet. The colonies containing more than 50 cells were counted. The surviving fraction of the radiated cells was normalized using the plating efficiencies of their corresponding untreated control. The data were fitted to the multi-target and linear-quadratic formulae, where survival (S) was related to dose (D) by the expression $S = 1 - (1 - e^{-D/D0})^N$ and $S = e^{-\alpha D - \beta D2}$ using SigmaPlot v.12 software. The survival curves were obtained, and the radiation-sensitivity parameters were calculated according to the published methods (12, 20).

MTT cell viability assay

SCC-61 and rSCC-61 response to Erlotinib. The viability of SCC-61 and rSCC-61 cells in response to treatment with the EGFR tyrosine kinase inhibitor, Erlotinib (also known as OSI-744 or Tarceva) was determined using the MTT (Roche) assay. The cells were trypsinized, resuspended in complete DMEM/F12 medium, and seeded in 96-well plates at a density of 5000/well in 50 μl. After overnight incubation at 37°C, the cells were treated with Erlotinib (50 μl) to the final concentrations of 0.5, 1, 10, 25, 50, and 100 μM, respectively, and incubated for 72 h at 37°C. After incubation, 10 μl MTT (5 mg/ml in PBS) was added to each well, and the plates were further incubated for 4 h at 37°C. At the end of the incubation, 100 μl solubilization solution was added to each well, and the absorbance was recorded at 570 nm. The resulting curves were fitted to a Hill equation with four parameters: $y = min + (max - min)/(1 + (x/IC50)^{(-Hillslope)}$ using SigmaPlot v.12 software. The 50% inhibitory concentration (IC50) was then determined as the Erlotinib concentration causing a 50% reduction in the cell viability. Effects of lipid raft inhibition on the response to Erlotinib and radiation in SCC-61. 5×10^4 cells/well were plated in 24-well plates and incubated overnight. Cells were then treated with 1 mM MβCD and 1 μM Lovastatin for 24 h. Next, cells were either irradiated with 0.5 and 2 Gy or treated with 2 μM Erlotinib. At 48 h post-treatment, MTT was added to the cultures, incubated for 4 h at 37°C, and processed as described earlier.

Cell-cycle analysis

The cell-cycle distribution of the SCC-61 and rSCC-61 cells was determined by flow cytometry. The SCC-61 and rSCC-61 cells were cultured in 100-mm dishes, radiated with a 2 Gy radiation dose, and incubated for 24 h at 37°C. The next day, the cells were trypsinized and resuspended in 1 ml propidium iodide staining solution (1 μg/ml) that was supplemented with 37 μg/ml RNase A and 0.06% NP-40. The data were collected using the Accuri C6 flow cytometer, and the cell-cycle distribution was analyzed by ModFit LT 3.2 DNA analysis software (Verity Software House).

Western blot analysis

At 24 h after radiation, the cells were lysed with the modified RIPA buffer and supplemented with protease and phosphatase inhibitors (cOmplete Tablets Mini and PhosSTOP, respectively from Roche). The lysates were incubated on ice for 1 h followed by centrifugation at 10,000 g for 10 min. The lysates were then normalized for their protein concentration across different treatment conditions and subjected to SDS-PAGE. The separated proteins were then transferred to a nitrocellulose membrane (0.45 μm; BioRad) and probed for the indicated proteins after overnight incubation with the corresponding primary antibodies (Cell Signaling) that was diluted in 1% BSA in TBS-Tween20 buffer followed by incubation with respective HRP-conjugated secondary antibodies. The Western blots were developed using Western Lightning Plus-ECL reagents followed by exposure to autoradiography film (Blue Ultra Autorad Film from GeneMate).

Total RNA isolation and semi-quantitative RT-PCR

Total RNA was extracted with RNeasy plus Micro kit (Qiagen). RNA (1 μg) was reverse transcribed with the SuperScript First-Strand cDNA Synthesis Kit (Invitrogen). Gene-specific primers were designed using Gene Runner

3.01 software. Semi-quantitative PCR was performed to examine the relative expression of Vimentin and E-cadherin in SCC-61 and rSCC-61. 18S amplification was used as an endogenous control. The primers and PCR conditions are shown in Supplementary Table S8.

Detection of reactive oxygen species

Subconfluent SCC-61 and rSCC-61 cells were cultured in 6-well plates and assayed for intracellular reactive oxygen species by imaging at 1 h after radiation (2 Gy) using 2.5 μM CM-H_2DCFDA (dichlorofluorescein [DCF]; Invitrogen). DCF was added for 10 min and incubated at room temperature before imaging. The cells were rapidly washed twice with 1 × PBS and visualized with an Arcturus PixCell II laser capture microscope under a 20 × objective. Both the fluorescence and the corresponding phase images were captured from at least two random fields per condition.

Glutathione assay

The concentration of total, oxidized, and reduced glutathione present in SCC-61 and rSCC-61 cells was determined using the o-phthalaldehyde-based glutathione assay kit (Biovision). Subconfluent cells were treated with 2 Gy radiation dose and incubated for 1 h post irradiation. The radiated and control SCC-61 and rSCC-61 cells were lysed using the assay buffer provided in the kit. The lysates were quickly added to the tubes containing prechilled perchloric acid (6N) and vortexed immediately. The samples were incubated on ice for 5 min and centrifuged at 13,000 *g* for 2 min to precipitate the proteins and separate the glutathione-containing supernatant. Ice-cold potassium hydroxide (KOH) (6N) was then added to the supernatant in order to neutralize the pH and to precipitate the perchloric acid. The supernatant was obtained by centrifugation at 13,000 *g*. For estimating total glutathione, oxidized glutathione (GSSG), and reduced glutathione (GSH), the samples were then diluted in the assay buffer in a 96-well plate with or without the glutathione reducing agent and reduced glutathione quencher (according to the manufacturer's instructions) and incubated with o-phthalaldehyde probe for 40 min at room temperature. Similar treatment was given to the reduced glutathione for generating the standard curve. The fluorescence was measured at λ_{ex} 340 nm and λ_{em} at 420 nm using the plate reader and reported as pmoles per μg of protein in assay.

Cell migration assay

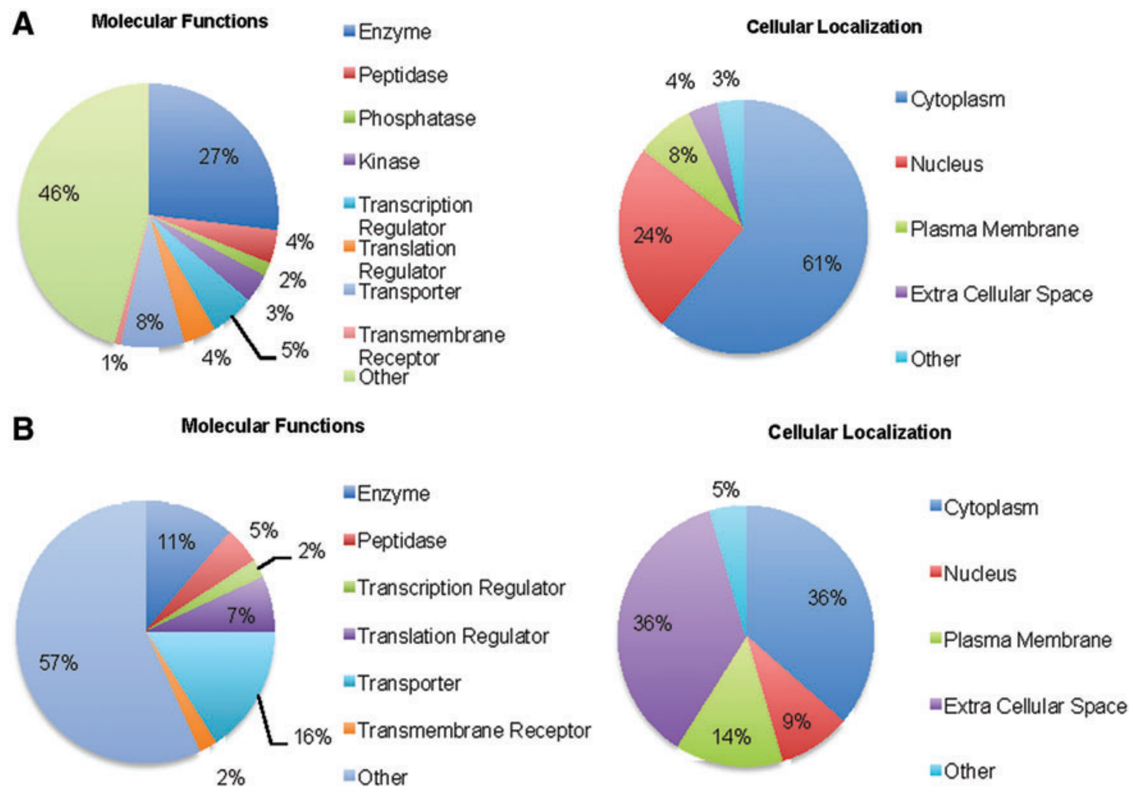
The transwell migration assay was performed following the manufacturer's protocol. Briefly, the SCC-61 and rSCC-61 cells were serum starved for about 16 h. The next day, the cells were dissociated, counted and 2.5×10^4 cells/well were added to the upper chamber of the transwell plates (BD Biosciences) in 500 μl serum-free medium. The lower chamber was filled with 500 μl complete DMEM/F12 medium. After incubation for 20 h at 37°C, the migrated cells were stained with 0.5% crystal violet and counted with the Olympus confocal microscope using a 20 × objective. Lactic acid assay and extracellular pH analysis were utilized. SCC-61 and rSCC-61 cells were dissociated with trypsin, counted, normalized, and cultured in six-well plates. After 4 h of incubation at 37°C and 5% CO_2 (zero time point), the cells were harvested for estimating the intracellular lactate levels. The culture medium was also collected for measuring the extracellular pH and lactate levels. The extracellular pH was measured with a laboratory pH meter (S20 SevenEasy from Metler Toledo). The intracellular and extracellular lactate levels were estimated using the Lactate Assay Kit II from BioVision according to the manufacturer's protocol. Total protein was estimated using the BCA assay (Thermo Scientific). The intracellular and extracellular lactate and extracellular pH were measured at different time intervals (4, 8, 24, 48, and 72 h). The lactate levels were determined and expressed as nmoles/μg/μl of the total protein.

Immunostaining of lipid rafts and EGFR

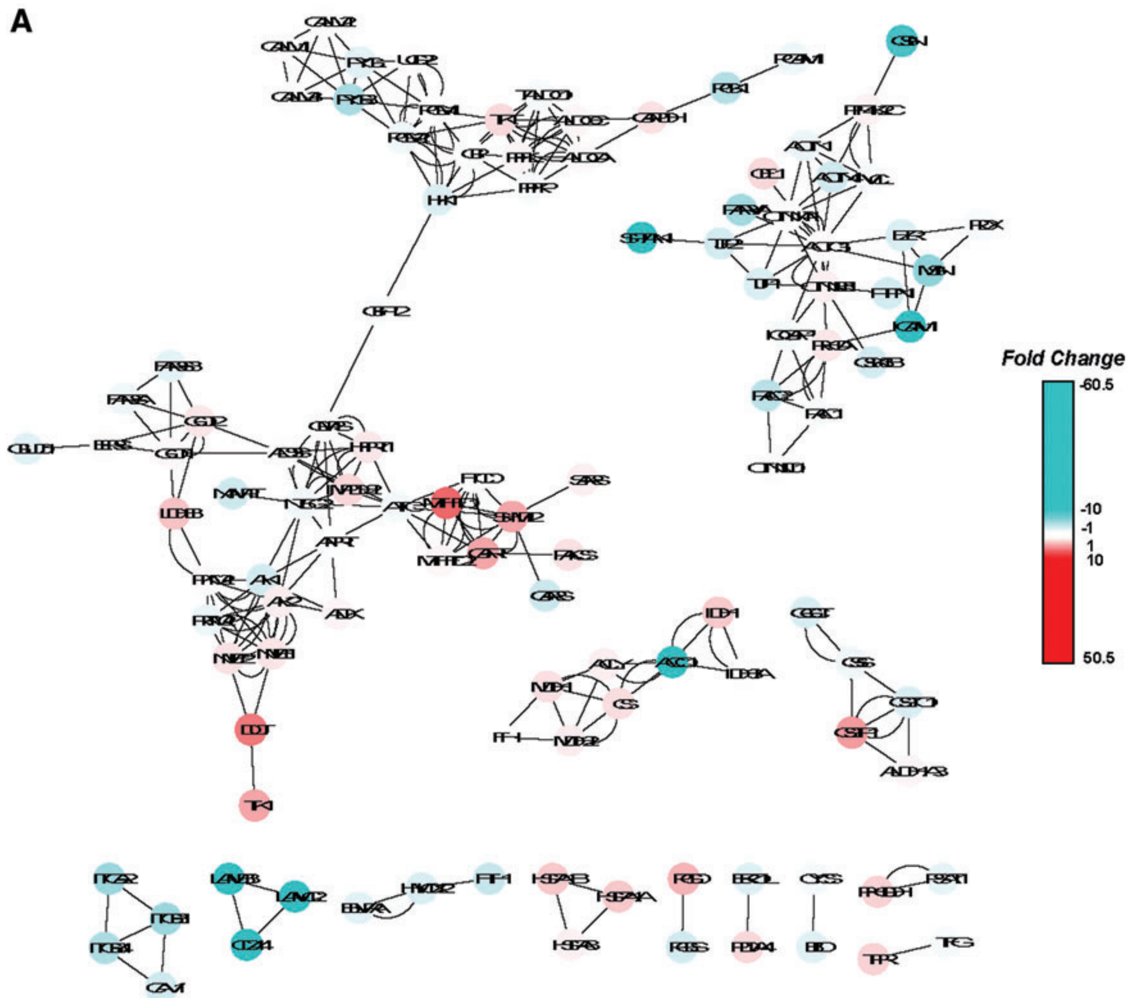
SCC-61/rSCC-61 comparison. SCC-61 and rSCC-61 cells were seeded in 1 ml microtek chambers at a density of 2×10^4 cells/ml and incubated overnight at 37°C and 5% CO_2. After overnight incubation, cells were treated with 2 Gy radiation. For lipid raft staining, the cells were incubated with 1 μg/ml of AlexaFluor594 conjugated CT-B for 15 min at 4°C before cell fixation. Cells were fixed in 4% paraformaldehyde for 15 min and permeabilized with 0.1 TritonX-100 followed by blocking with 8% BSA for 1 h at 4°C. The cells were then incubated with 1:500 dilution of AlexaFluor488 conjugated anti-EGFR antibody (Millipore) for 1 h at 4°C, washed, and mounted using Fluoromount (Sigma) for imaging. Imaging was performed using a Zeiss LSM710 confocal microscope and a 40 × objective. The images were processed using the LSM image browser. Inhibition of lipid rafts was conducted in SCC-61 by MβCD, Lovastatin, and polyethylene glycol (PEG)-catalase. SCC-61 cells were seeded in 1 ml microtek chambers at a density of 2×10^4 cells/ml and incubated overnight at 37°C and 5% CO2. After overnight incubation, cells were treated with (i) 1 mM MβCD, (ii) 2 μM Lovastatin, (iii) 1 mM MβCD plus 2 μM Lovastatin, and (iv) PEG-catalase (2 and 20 U) in 1 ml medium. After 24 h treatment, the cells were stained with AlexaFluor594 conjugated CT-B and imaged as described earlier.

γH2AX Immunofluorescence

SCC-61 and rSCC-61 cells were seeded in 1 ml microtek chambers at a density of 2×10^4 cells/ml and incubated overnight at 37°C and 5% CO2. After overnight incubation, cells were treated with 2 Gy radiation. At 3 and 24 h postirradiation, cells were washed with cold PBS and fixed in 4% paraformaldehyde for 15-min, washed with 100% methanol for 1 min at 20°C, and permeabilized with 0.1 TritonX-100 followed by blocking with 8% BSA for 1 h at room temperature. The cells were incubated with 1:500 dilution of γH2AX overnight at 4°C followed by washing with PBS, incubation with 1:500 dilution of 2° anti-rabbit Alexa Fluor 488 Conjugate for 1 h at room temperature, and 10 min incubation with 1:100 Topro-3-iodide before mounting with Fluoromount (Sigma). Imaging was performed using a Zeiss LSM710 confocal microscope and a 63 × objective.



SUPPLEMENTARY FIG. S1. Cellular localization and molecular functions of the 965 proteins identified in the proteomic analysis (A) and the subset of 45 proteins with less than 0.01 expression ratio in rSCC-61 *versus* SCC-61 cells (B).



SUPPLEMENTARY FIG. S2. Functional network analysis-KEGG interaction subnetwork. (A) KEGG interaction subnetwork. Cytoscape was used to analyze MS data using interactions imported from the KEGG database. The figure shows subnetworks created from the global KEGG network by selecting all nodes (proteins) with a significant fold change (≥ 2.0 or ≤ -2.0) along with their first neighbors. Node colors represent fold change, where red is upregulation and blue is downregulation as indicated by the fold-change bar. DAVID analysis of the Uniprot IDs of these significantly regulated nodes identified several over-represented ($p < 0.05$) KEGG pathways in this network. The subnetwork that had a high density of proteins involved in the regulation of actin cytoskeleton and focal adhesion is depicted on the right. **(B)** List of significantly over-represented KEGG pathways and their corresponding P values. DAVID, Database for Annotation, Visualization and Integrated Discovery; KEGG, Kyoto Encyclopedia of Genes and Genomes.

B

KEGG Pathway	P value
hsa00030:Pentose phosphate pathway	1.34E-10
hsa00010:Glycolysis / Gluconeogenesis	1.24E-09
hsa04520:Adherens junction	2.72E-07
hsa00020:Citrate cycle (TCA cycle)	5.23E-07
hsa04670:Leukocyte transendothelial migration	5.00E-06
hsa00230:Purine metabolism	1.88E-05
hsa00630:Glyoxylate and dicarboxylate metabolism	2.04E-05
hsa00670:One carbon pool by folate	2.91E-05
hsa04810:Regulation of actin cytoskeleton	6.16E-05
hsa04510:Focal adhesion	1.03E-04
hsa00052:Galactose metabolism	3.60E-04
hsa00500:Starch and sucrose metabolism	4.97E-04
hsa00520:Amino sugar and nucleotide sugar metabolism	6.42E-04
hsa00480:Glutathione metabolism	1.28E-03
hsa05200:Pathways in cancer	2.52E-03
hsa00250:Alanine, aspartate and glutamate metabolism	6.55E-03
hsa00051:Fructose and mannose metabolism	9.12E-03
hsa04530:Tight junction	1.67E-02
hsa00970:Aminoacyl-tRNA biosynthesis	1.75E-02
hsa00860:Porphyrin and chlorophyll metabolism	4.63E-02
hsa00270:Cysteine and methionine metabolism	4.99E-02

SUPPLEMENTARY FIG. S2. (Continued).

Supplementary Table S1. List of Calculated Values of D_0, α, and β Parameters for the Clonogenic Assays in SCC-61 and rSCC-61

Cell lines	D_0	N	α	B	PE	SF_1	SF_2	SF_4	SF_6
rSCC-61	2.04	2.20	0.13	0.04	49.0%	0.88	0.64	0.30	0.10
SCC-61	1.32	1.95	0.27	0.09	28.8%	0.71	0.39	0.09	0.03

In the multi-target model, D_0 parameter indicates the amount of radiation required to reduce the survival fraction to $\sim$0.37. In the linear-quadratic model, there are two components of cell killing: One is proportional to the IR dose (αD), and the other is proportional to the square of the IR dose (βD^2).

PE, plating efficiency; SF, surviving fraction.

Supplementary Table S2. List of Proteins with Expression Ratio <0.01 in rSCC-61 and SCC-61 Generated by the SILAC Proteomic Analysis

Proteins
Adenosine deaminase
Afamin
Alpha-1B-glycoprotein
Alpha-2-macroglobulin
Alpha-actinin-3
Alpha-aminoadipicsemialdehyde dehydrogenase
Ankyrin-3
Apolipoprotein E
BTB/POZ domain-containing protein KCTD12
Desmoglein-3
Eukaryotic peptide chain release factor subunit
Fibronectin type III domain-containing protein 3B
HEAT repeat-containing protein 2
Helicase SKI2W
Hematopoietic lineage cell-specific protein
Hemoglobin subunit alpha
Hemoglobin subunit epsilon
HLA class I histocompatibility antigen, A-25 alpha chain
Insulin-like growth factor 2 mRNA-binding protein 2
Insulin-like growth factor 2 mRNA-binding protein 3
Integrin alpha-6
Inter-alpha-trypsin inhibitor heavy chain H2
Involucrin
Lactotransferrin
Neutrophil gelatinase-associated lipocalin
Normal mucosa of esophagus-specific gene 1 protein
Nuclear pore complex protein Nup205
Plasminogen activator inhibitor
Plasminogen activator inhibitor 2
Plastin-2
Pregnancy zone protein
Protein S100-A7
Protein S100-A8
Protein S100-A9
Protein-glutamine gamma-glutamyltransferase 2
Putative FAM75-like protein FLJ44082
Serum albumin
Serum amyloid A protein
Succinate dehydrogenase [ubiquinone] flavoprotein subunit, mitochondrial
Thrombomodulin
Thrombospondin-1
Thyroxine-binding globulin O
Translocon-associated protein subunit alpha
Trypsin-3
Vitronectin

SILAC, stable isotope labeling with amino acids in cell culture.

Supplementary Table S3. List of the Top Upregulated Proteins in rSCC-61 Generated by the SILAC Proteomic Analysis

Proteins	*Fold change (rSCC-61/SCC-61)*
Keratin Type II cytoskeletal 8 (KRT8)	50.5
Keratin Type I Cytoskeletal 18 (KRT18)	13.8
Keratin Type I Cytoskeletal 17 (KRT17)	8.9
Fatty Acid Synthase (FASN)	6.8
C-1-tetrahydrofolate synthase	6.3
Deoxyuridine 5′-triphosphate nucleotidohydrolase (DUT)	5.6
Periplakin	4.8
Structural maintenance of chromosome flexible hinge domain containing protein 1	4.7
Protein S100-A16	4.5
Glutathione S-transferase pi (GSTpi)	4.4
Trifunctional purine biosynthetic protein adenosine-3	4.0
Serine hydroxymethyltransferase (SHMT2)	4.0
Activator of 90 kDa heat shock protein ATPase homolog 1	3.9

Supplementary Table S4. Fold Change in a Subset of Proteins Predicted to Decrease Cell Death and Apoptosis in rSCC-61 Obtained from the SILAC Proteomic Analysis

Protein	*Fold change (rSCC-61/SCC-61)*
Keratin Type II cytoskeletal 8 (KRT8)	50.5
Keratin Type I cytoskeletal 18 (KRT18)	13.8
Fatty acid synthase (FASN)	6.8
Glutathione S-transferase pi (GSTpi)	4.4
Activator of 90 kDa heat shock protein ATPase homolog 1 (AHSA1)	3.9
A-kinase anchor protein 12 (AKAP12)	2.9
Desmoplakin (DSP)	2.9
Serine/arginine-rich splicing factor 5 (SRSF5)	2.5
Proliferation-associated 2G4, 38 kDa (PA2G4)	2.5
Peroxiredoxin (PRDX3)	2.1
Serpin peptidase inhibitor, clade B (ovalbumin), member 5 (SERPINB5)	−30.6
Intercellular adhesion molecule 1 (ICAM1)	−19.9
SH3-domain kinase binding protein 1 (SH3KBP1)	−15.9
Reticulon 4 (RTN4)	−14.9
CD44 molecule (Indian blood group)	−11.6
Microtubule-associated protein 1 light chain 3 alpha (MAP1LC3A)	−6.0
FK506 binding protein 1A, 12 kDa (FKBP1A)	−5.3
Integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12) (ITGB1)	−4.7
BH3 interacting domain death agonist (BID)	−2.1

Supplementary Table S5A. Fold Change in a Subset of Proteins Involved in DNA Replication and Base-Excision Repair Obtained from the SILAC Proteomic Analysis

Proteins	*Fold change (rSCC-61/SCC-61)*
Minichromosome maintenance complex component 7 (MCM7)	2.9
Minichromosome maintenance complex component 2 (MCM2)	2.8
Minichromosome maintenance complex component 6 (MCM6)	2.6
Minichromosome maintenance complex component 3 (MCM3)	2.7
Poly (ADP-ribose) polymerase 1 (PARP1)	2.5
APEX nuclease (multifunctional DNA repair enzyme) 1 (APEX1)	2.5
Ligase I, DNA, ATP-dependent (LIG1)	2.5
High mobility group box 1 (HMGB1)	2.3

Supplementary Table S5B. Fold Change in a Subset of Proteins Involved in ECM-Receptor Interaction Obtained from the SILAC Proteomic Analysis

Proteins	*Fold change (rSCC-61/SCC-61)*
Laminin, beta 3 (LMB3)	−20.0
Laminin, gamma 2 (LMC2)	−18.6
CD44 molecule (Indian blood group)	−11.6
Integrin, beta 1 (ITGB1)	−4.8
Integrin, alpha 2 (ITGA2)	−4.8
Integrin, beta 4 (ITGB4)	−4.0

ECM, extracellular matrix.

Supplementary Table S5C. Fold Change in a Subset of Proteins Involved in Regulation of Cell Cycle Obtained from the SILAC Proteomic Analysis

Proteins	*Fold change (rSCC-61/SCC-61)*
Minichromosome maintenance complex component 7 (MCM7)	2.9
Glycogen synthase kinase 3 beta (GSK3B)	−2.6
Structural maintenance of chromosomes 1A (SMC1A)	2.3
Minichromosome maintenance complex component 2 (MCM2)	2.8
Protein kinase, DNA-activated, catalytic polypeptide (PRKDC)	2.3
Minichromosome maintenance complex component 6 (MCM6)	2.6
Minichromosome maintenance complex component 3 (MCM3)	2.8

Supplementary Table S5D. Fold Change in a Subset of Proteins Involved in Focal Adhesion Obtained from the SILAC Proteomic Analysis

Proteins	*Fold change (rSCC-61/SCC-61)*
Laminin, beta 3 (LMB3)	−20.0
Laminin, gamma 2 (LMC2)	−18.6
Integrin, beta 1 (ITGB1)	−4.8
Integrin, alpha 2 (ITGA2)	−4.8
Parvin, alpha (PARVA)	−4.5
Integrin, beta 4 (ITGB4)	−4.0
Ras-related C3 botulinum toxin substrate 2 (RAC2)	−3.5
Caveolin 1 (CAV1)	−2.7
Actinin alpha 4 (ACTN4)	−2.7
Glycogen synthase kinase 3 beta (GSK3B)	−2.6
Ras homolog family member A (RHOA)	1.8
catenin (cadherin-associated protein), beta 1 (CTNNB1)	1.5

Supplementary Table S5E. Fold Change in a Subset of Proteins Involved in Regulation of Actin Cytoskeleton Obtained from the SILAC Proteomic Analysis

Proteins	*Fold change (rSCC-61/SCC-61)*
Gelsolin (GELS)	−8.8
Moesin (MOES)	−5.4
Integrin, beta 4 (ITGB4)	−4.0
Integrin, alpha 2 (ITGA2)	−4.8
Integrin, beta 1 (ITGB1)	−4.8
Ras-related C3 botulinum toxin substrate 2 (RAC2)	−3.5
Actinin alpha 4 (ACTN4)	−2.7
Ezrin (EZR)	−2.4
Actinin alpha 1(ACTN1)	−1.7
Cofilin-1 (COF1)	2.3
Ras homolog family member A (RHOA)	1.8

Supplementary Table S6. Fold Change in Proteins Predicted to Decrease ROS in rSCC-61 Obtained from the SILAC Proteomic Analysis

Proteins	*Fold change (rSCC-61/SCC-61)*
Peroxiredoxin 1	2.2
Peroxiredoxin 3	2.1
Glutathione S-transferase pi (GST pi)	4.4

ROS, reactive oxygen species.

Supplementary Table S7. Fold Change in Expression of Subset of Proteins Predicted to Decrease DNA Damage in rSCC-61 Obtained from the SILAC Proteomic Analysis

Protein	*Fold change (rSCC-61/SCC-61)*
Glutathione S-transferase pi 1 (GSTP1)	4.4
Poly (ADP-ribose) polymerase 1 (PARP1)	2.5
Ligase I, DNA, ATP-dependent (LIG1)	2.5
Protein kinase, DNA-activated, catalytic polypeptide (PRKDC)	2.3
Nucleophosmin (NPM1)	2.3
Peroxiredoxin 1 (PRDX1)	2.2
MutS homolog 6 (MSH6)	1.9
Nucleoside diphosphate kinase 1 (NME1)	1.8

Supplementary Table S8. Primers and PCR Conditions for Vimentin and E-cadherin

Gene	*5′-3′ sequence*	*Length (bp)*
E-cadherin	F: CTCCCATCAGCTGCCCAGAA R: TCAGGATCTTGGCTGAGGATG	454
Vimentin	F: AAGCAGGAGTCCACTGAGTAC R: GGTATCAACCAGAGGGAGTGA	372
18S	F: GTGGTGTTGAGGAAAGCAGACA R: TGATCACACGTTCCACCTCATC	98

PCR conditions: 95°C for 5 min; 28 cycles of 95°C for 30 s, 54°C for 30 s and 72°C for 30 s; and a final extension step for 5 min at 72°C.
F, forward primer; R, reverse primer.