Am J Cancer Res 2019;9(6):1201-1211
www.ajcr.us /ISSN:2156-6976/ajcr0090118

*Original Article*

# Glutathione reductase (*GSR*) gene deletion and chromosome 8 aneuploidy in primary lung cancers detected by fluorescence in situ hybridization

Mohamed Baity[1], Li Wang[2], Arlene M Correa[2], Xiaoshan Zhang[2], Ran Zhang[2], Apar Pataer[2], Shuhong Wu[2], Qing H Meng[3], Mara B Antonoff[2], Wayne L Hofstetter[2], Reza J Mehran[2], David C Rice[2], Jack A Roth[2], Boris Sepesi[2], Stephen G Swisher[2], Ara A Vaporciyan[2], Garrett L Walsh[2], Ming Zhao[1], Jun Gu[1], Bingliang Fang[2]

*[1]School of Health Professions, Departments of [2]Thoracic and Cardiovascular Surgery, [3]Laboratory Medicine, The University of Texas MD Anderson Cancer Center, Houston, TX 77030, USA*

Received December 19, 2018; Accepted January 4, 2019; Epub June 1, 2019; Published June 15, 2019

**Abstract:** Our recent study demonstrated that cancer cells with compromised glutathione homeostasis, including reduced expression of the glutathione reductase (*GSR*) gene, were selectively killed by inhibition of thioredoxin reductase. The human *GSR* gene is located on chromosome 8p, a region often lost in lung and other cancers. However, whether *GSR* is altered in primary lung cancer remains unknown. To analyze alterations of *GSR* in lung cancer, we performed fluorescence in situ hybridization with probes for *GSR* and the chromosome 8 centromere (CEP8) in 45 surgical specimens of primary lung cancer, including 24 lung adenocarcinomas, 10 squamous cell carcinomas, 8 neuroendocrine cancers, and 3 small cell lung cancers. Twenty-five surgically resected normal lung tissue specimens from these lung cancer patients were used as a controls. The signal ratio of *GSR* to CEP8 per cell was used to identify gain or loss of *GSR*. *GSR* loss was detected in 6 of 24 (25%) adenocarcinoma specimens and 5 of 10 (50%) squamous cell carcinoma specimens, but not in neuroendocrine cancer or small cell lung cancer specimens. We also found that 19 of 45 (42%) specimens had chromosome 8 aneuploidy (more or less than 2 signals for CEP8), including 8 with both aneuploidy and *GSR* deletion. Chromosome 8 aneuploidy was detected in all types of lung cancer analyzed. Univariate and multivariable logistic regression analyses indicated that male patients had an increased risk of *GSR* deletion (hazard ratio [HR] = 4.77, 95% confidence interval [CI] = 1.00-22.86, *P* = 0.051), and patients who had undergone preoperative radiation therapy or had a self-reported history of cigarette smoking had an increased risk of chromosome 8 aneuploidy (preoperative radiation: HR = 18.63, 95% CI = 0.90-384.17, *P* = 0.058; smoking: HR = 7.59, 95% CI = 0.86-66.75, *P* = 0.068), although the *p* values did not reach significance. Because *GSR* deficiency and chromosome 8 aneuploidy have implications in targeted therapy and/or immunotherapy for cancer, they might serve as predictive biomarkers for precision therapy of lung cancers.

**Keywords:** Chromosome 8p, glutathione reductase, gene deletions, gene copy number variations, aneuploidy

## Introduction

The short arm of chromosome 8 at bands 12 to 23 (8p12-23) is frequently altered in human cancers [1-3], including lung cancers [4, 5]. The most frequent alterations in this region are deletions and loss of heterozygosity, although gene amplifications have been reported [6]. Copy number losses of 8p have frequently been detected in early-stage lung adenocarcinoma and squamous cell carcinoma [4, 7]. Chromosome arm 8p losses have been shown to be associated with tumor progression and resistance to chemotherapy and radiotherapy [2, 8], leading to adverse clinical outcomes [9]. Several genes in this region have been investigated as potential tumor suppressor genes or oncogenes, including *NRG1* [10], *DLC1* [11], *GATA4* [12], and the genes encoding the tumor necrosis factor-related apoptosis-inducing ligand receptors DR4 (*TNFRSF10A*) and DR5 (*TNFRSF10B*) [13, 14].

The gene *GSR*, which encodes glutathione (GSH) reductase and plays a critical role in redox homeostasis and cellular defense against oxidative stress [15], is located at 8p12. GSH is a major intracellular antioxidant that is crucial

in the maintenance of intracellular redox homeostasis, signal transduction, the metabolism of xenobiotics, and the balance of thiols. Catalyzed by GSH peroxidases, GSH reacts readily with reactive oxygen species and is oxidized to GSH disulfide (GSSG) under oxidative stress. GSR catalyzes recycling of GSSG back to GSH by using reduced nicotinamide adenine dinucleotide phosphate as an electron donor. Alteration of the intracellular GSH/GSSG ratio or depletion of GSH is often associated with the initiation or progression of apoptosis [16, 17].

Because GSH plays a critical role in cancer progression and treatment responses [18, 19], GSR deficiency is expected to affect cancer patients' clinical outcomes. Increased expression of GSR was associated with resistance to temozolomide [20] and to oxidative stress-induced cell death [21]. Our recent study revealed that reduced expression of the genes involved in GSH homeostasis, including *GSR* and *GCLC*, sensitized cancer cells to small molecule inhibitors of thioredoxin reductase, such as auranofin [22]. Nevertheless, little is known about genomic alterations in this gene in primary human cancers.

To investigate whether *GSR* is altered in human cancer, we searched The Cancer Genome Atlas database (www.cbioportal.org) for copy number alterations of *GSR* and genes located at 8p21-23 (*TNFRSF10A*, *TNFRSF10B*, *DLC1*, and *GATA4*) in several types of cancers. We found that *GSR* deletions occur in human lung, breast, liver, and prostate cancers, at rates ranging from 4% to 10% and that most *GSR* deletions co-occur with deletions of genes located at 8p21-23. We then analyzed *GSR* copy number changes in primary lung cancer samples using fluorescence in situ hybridization (FISH). We detected *GSR* deletion in 25% of adenocarcinoma samples and 50% of squamous cell carcinoma samples, but not in neuroendocrine cancer or small cell lung cancer samples. Moreover, we found that 42% of lung cancer samples had chromosome 8 aneuploidy, which was detected in all types of lung cancer analyzed.

## Materials and methods

### *Human cell and tissue samples*

Normal human lymphocytes were obtained from blood samples from healthy donors and cultured in RPMI 1640 medium supplemented with 10% fetal bovine serum, 100 units/mL penicillin, and 100 μg/mL streptomycin. Cells were maintained in a humidified atmosphere containing 5% $CO_2$ at 37°C. To prepare metaphase cells for FISH analysis, demecolcine (0.1 μg/mL) was added to the culture medium 40 minutes before cell harvest. Fresh lung tissue and cancer samples were collected during 2015 and 2017 from surgically resected specimens. The clinical data, including pathological diagnosis and survival data, were collected from patient records. The research protocols for the use of clinical specimens and data in this study were approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center. All clinical samples and data were collected with informed consent from the patients.

### *Preparation of FISH probes*

A Vysis CEP8 (D8Z2) SpectrumGreen FISH probe for the human chromosome 8 centromere was purchased from Abbott Molecular (Abbott Park, IL). The bacterial artificial chromosome (BAC) clone (RP11-411F21) containing the entire *GSR* gene was obtained from Children's Hospital Oakland Research Institute. BAC clone DNA was isolated from host bacteria using PureLink HiPure Plasmid Filter Purification Kits (catalog #K210002; Thermo Fisher Scientific, Inc., Waltham, MA) and then labeled with SpectrumOrange-dUTP using the nick translation kit (catalog #07J00-001; Abbott Molecular) following the manufacturer's protocol. The prepared probe was validated by its hybridization to chromosome arm 8p in metaphases of normal human lymphocytes, with CEP8 Spectrum Green as a control for the chromosome 8 centromere. The validated probe was stored at -20°C until analysis.

### *FISH analysis*

Fresh tissue samples were briefly touched to the center portion of Superfrost microscope slides (Thermo Fisher Scientific, Inc.,) 5 times. After air drying for 5 minutes, the cells on the slides were fixed with methanol/glacial acetic acid (3:1) twice, each time for 10 minutes. The slides were stored at -80°C until FISH analysis. Then, the tissue touch preparation slides were pretreated in a methanol/glacial acetic acid fixative series: 1:1 for 20 minutes, 1:1 for 10 minutes, and 3:1 for 10 minutes. The slides

were then processed with 2× standard saline citrate (2×SSC, 300 mmol/L sodium chloride and 30 mmol/L sodium citrate) at 37°C for 30 minutes, dehydrated with an ethanol series (70%, 85%, and 100% for 2 minutes each), and air dried. A total of 6 μL of the FISH probe mixture with 3 μL home-brew SpectrumOrange GSR probe and 3 μL SpectrumGreen CEP8 (D8Z2) probe working solution (prepared by mixing 1 μL CEP8 probe with 2 μL H2O and 7 μL CEP hybridization buffer from Abbott Moecular, #07J36-001) was placed on each hybridization area. A coverslip (18×18 mm) was placed over the hybridization area and sealed to the slide with rubber cement. The slide and the probe were first incubated for 15 minutes at 37°C, then co-denatured for 5 minutes at 75°C, and then incubated at 37°C for at least 12 hours in a ThermoBrite denaturation-hybridization system (Abbott Molecular). The rubber cement and coverslip were removed, and the slides were washed in 2×SSC/0.3% Nonidet P-40 at 72°C for 1 minute, followed by 2×SSC/0.1% Nonidet P-40 at room temperature for 1 minute. Twenty microliters of DAPI II counterstain (Abbott Molecular) was then added to the hybridization area, which was covered with a 22×22 mm coverslip. The sample was then subjected to standard fluorescence microscopy analysis for hybridization signals. For each sample, 200 interphase cells were analyzed for the number of red (GSR) and green (CEP8) signals. Two individuals independently scored the slides; each recorded the signal patterns encountered in 100 cells.

*Statistical analysis*

Each cell was counted individually for red and green signals. Descriptive statistics were used for the percentages of cells with various red and green signals. A red/green ratio or more than 1 indicated *GSR* copy number gain and a ratio of less than 1 indicated copy number loss. Aneuploidy was defined as any number of CEP8 (green) signals other than two. On the basis of means and standard deviations observed in cells from normal lung tissues, we considered there to be no alteration if more than 92% of the cells had 2 green and 2 red signals. An abnormal pattern was defined as 8% or more of interphase cells with any pattern of aneuploidy or *GSR* copy number variations.

Associations between categorical variables were analyzed using the Pearson chi-square or Fisher exact test. For continuous variables, we used the Mann-Whitney U test. Two univariate logistic regression analyses were performed, using *GSR* deletion or aneuploidy as the outcomes, with a significance level of $P < 0.05$. Covariates that were significant at $P < 0.25$ were included in multivariable logistic regression. Backward stepwise Wald elimination at $P = 0.10$ was used to establish the final model. Specimens from patients with incomplete data were excluded from the multivariable analysis. Survival functions were calculated using the Kaplan-Meier method, and differences were assessed with the log-rank test. Univariate and multivariable Cox regression models using death or recurrence as the endpoints were performed, using the same strategy described above for the multivariable logistic regression analyses. All statistical analyses were performed using SPSS V24.0 (IBM, Inc., Armonk, NY).

## Results

*Validation of the GSR FISH probe*

Because chromosome arm 8p is frequently altered in human cancers [1-3], and because the GSH/GSR system may play role in cancer progression and treatment response, we queried The Cancer Genome Atlas database (www.cbioportal.org) for copy number variations of *GSR* (located at 8p12) and genes located at 8p21-23 (*TNFRSF10A*, *TNFRSF10B*, *DLC1*, and *GATA4*) that have been found to be deleted in some human cancers. *GSR* was deleted in about 4% of human lung adenocarcinoma and squamous cell carcinoma samples and in about 5% to 10% of human breast, liver, and prostate cancer samples (Figure S1). Most *GSR* deletions were concurrent with deletions of the other genes at 8p21-23, suggesting that *GSR* genomic alteration is concurrent with chromosome 8p deletions in human cancers.

To determine copy number variations of *GSR* in human lung cancer, we prepared a FISH probe covering the entire *GSR* gene. The probe was validated by its specific hybridization to chromosome 8p adjacent to the CEP8 probe in the chromosomes of normal human lymphocytes

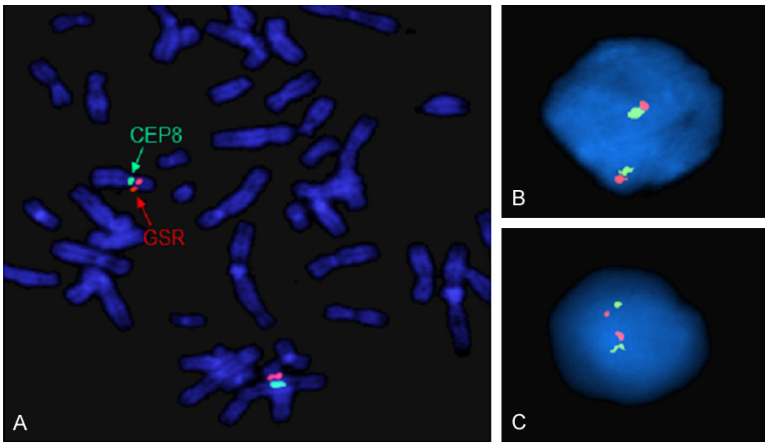


**Figure 1.** Validation of the *GSR* fluorescence in situ hybridization (FISH) probe. FISH analysis was performed in normal human lymphocytes (A) and cells from human lung tissues (B and C) at metaphase for *GSR* (red) and CEP8 (green). When the cells were in normal metaphase, *GSR* and CEP8 hybridized to the same chromosome, whereas when cells were in normal interphase, the 2 probes were located close to each other.

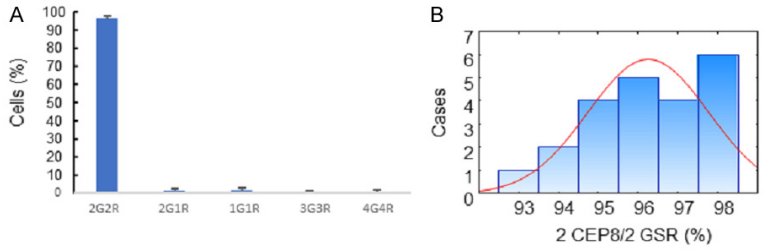


**Figure 2.** *GSR*/CEP8 fluorescence in situ hybridization signals in 25 normal lung tissue samples from patients with lung cancer. The lung tissues were obtained from sites at least 5 cm away from the tumor margins. *GSR* (red) and CEP8 (green) signals were counted in 100 cells per sample. (A) Percentage of cells with different green (G) and red (R) signal patterns. (B) Distribution of cells with 2 CEP8 and 2 *GSR* (normal diploid) signals.

(**Figure 1A**), indicating that the probe could be used to detect 8p copy number variation at the region where *GSR* is located.

*GSR copy number detected by FISH in cells from normal lung tissues*

We collected normal lung tissues from surgical specimens from 25 patients with non-small cell lung cancer. Among these specimens, 17 had matched tumor tissues and 8 were normal tissue only. Thirteen of the patients had adenocarcinoma, 9 had squamous cell carcinoma, and 3 had neuroendocrine cancer. The normal tissues were obtained from sites at least 5 cm away from the tumor margin. Examples of the FISH signal in cells from normal lung tissues are shown in **Figure 1B** and **1C**. Most normal cells had 2 green and 2 red signals that occurred near each other. We counted the number of red and green FISH signals in 100 cells per specimen. **Figure 2** shows the number of cells from normal lung tissues with different combinations of green and red signals. About 96% (mean) of normal cells had 2 green and 2 red signals (96.16% ± 1.43%, range 93%-98%). Between 0.4% and 1.6% of cells had 2 green and 1 red, 1 green and 1 red, 3 green and 3 red, or 4 green and 4 red signals. This result is consistent with the range of test values expected to occur in 95% of healthy individuals, known as the reference range, as defined by the American College of Medical Genetics FISH Guidelines (www.acmg.net, accessed September 2010).

*GSR copy number detected by FISH in cells from lung cancer tissues*

We analyzed *GSR* copy number variations using FISH analysis in 45 lung cancer specimens. Among these specimens, 24 were adenocarcinomas, 10 were squamous cell carcinomas, 8 were neuroendocrine cancers, and 3 were small cell lung cancers. On the basis of the number of signals detected for CEP8 (green) and *GSR* (red) per cell, we found 3 major abnormal patterns in cells derived from cancer tissues: (1) *GSR* number copy loss (cells had 2 green signals but only 1 red signal); (2) aneuploidy (cells had more or less than 2 green signals but an equal number of red and green signals); and (3) aneuploidy with *GSR* deletion (cells had more or less than 2 green signals and fewer red signals than green signals). Most aneuploid cells had more than 2 green signals. Examples of these abnormal patterns are shown in **Figure 3**. Among the 45 lung cancer specimens analyzed, 3 (7%) had

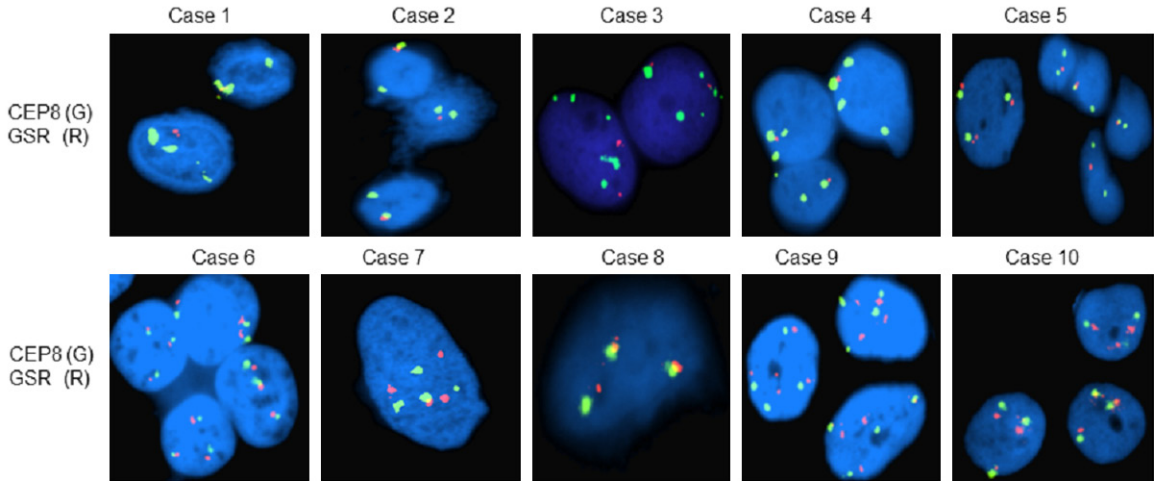


**Figure 3.** Examples of abnormal fluorescence in situ hybridization signals in lung cancer specimens. Cases 1 and 2 had 2 green signals and 1 red signal per cell, indicating *GSR* deletion. Cases 3 and 4 had more than 3 green signals per cell and fewer red signals than green signals, indicating aneuploidy with *GSR* deletion. Case 5 had aneuploid cells and cells with *GSR* deletion. Cases 6-10 had aneuploid cells.

**Table 1.** *GSR* deletion and chromosome 8 aneuploidy in primary lung cancer specimens (n = 45)

| Histologic subtype | No. of specimens | | | | |
|---|---|---|---|---|---|
| | No alteration | Alteration | | | Total |
| | | *GSR* deletion | Aneuploidy and *GSR* deletion | Aneuploidy | |
| Adenocarcinoma | 13 | 2 | 4 | 5 | 24 |
| Squamous cell carcinoma | 4 | 1 | 4 | 1 | 10 |
| Neuroendocrine cancer | 6 | 0 | 0 | 2 | 8 |
| Small cell lung cancer | 0 | 0 | 0 | 3 | 3 |
| Total | 23 | 3 | 8 | 11 | 45 |
| Percentage | 51% | 7% | 18% | 24% | 100% |

GSR gene deletion, 8 (18%) had aneuploidy plus *GSR* deletion, and 11 (24%) had aneuploidy. **Table 1** summarizes the distributions of these abnormalities among different histologic subtypes.

*Clinical associations with abnormal FISH signals*

We performed statistical analysis to determine whether *GSR* deletion and/or aneuploidy detected by FISH was associated with any clinical or demographic parameters. Univariate and multivariable logistic regression analyses indicated that sex may be associated with *GSR* deletion. Male patients had a higher risk of *GSR* deletion ($P$ = 0.051) than did female patients (**Table 2**). *GSR* deletion was observed in both adenocarcinoma and squamous cell carcinoma samples, however, we did not observe *GSR* deletion in neuroendocrine cancer or small cell lung cancer samples.

Aneuploidy was detected in all histologic subtypes of primary lung cancer. Multivariable logistic regression analysis indicated that the risk for aneuploidy may be higher in lung cancer patients who have undergone preoperative radiation therapy ($P$ = 0.058) and in those with a history of cigarette smoking ($P$ = 0.068; **Table 3**), although in both cases the $p$ values did not reach significance.

We also determined whether *GSR* deletion or aneuploidy in lung cancer was associated with the duration of disease-free survival or the probability recurrence. Neither *GSR* deletion nor aneuploidy was significantly associated with disease-free survival or recurrence probability (Figure S2).

**Table 2.** Regression analyses showing association of *GSR* deletion and clinical parameters

| Parameter | No. of cases | GSR deletion No. (%) | Hazard ratio | 95% confidence interval | *P* |
|---|---|---|---|---|---|
| Univariate logistic regression | | | | | |
| Sex | | | | | |
| Female (reference) | 27 | 4 (15) | 1.00 | | |
| Male | 18 | 7 (39) | 3.66 | 0.88-15.19 | 0.074 |
| Age in years (continuous) | 45 | | 1.03 | 0.96-1.11 | 0.446 |
| Histologic subtype | | | | | 0.204 |
| Adenocarcinoma (reference) | 24 | 6 (25) | 1.00 | | |
| Squamous cell carcinoma | 10 | 5 (50) | 2.40 | 0.48-11.97 | 0.286 |
| Others* | 11 | 0 (0) | 0.27 | 0.03-2.58 | 0.257 |
| Pathologic T category | | | | | 0.401 |
| T1 (reference) | 15 | 2 (13) | 1.00 | | |
| T2 | 21 | 7 (33) | 3.25 | 0.57-18.58 | 0.185 |
| T3-T4 | 9 | 2 (22) | 1.86 | 0.21-16.18 | 0.575 |
| Pathologic N category | | | | | |
| N0 (reference) | 28 | 8 (29) | 1.00 | | |
| N1-N2 | 17 | 3 (18) | 0.54 | 0.12-2.38 | 0.412 |
| Pathologic M category | | | | | |
| M0 (reference) | 39 | 9 (23) | 1.00 | | |
| M1 | 6 | 2 (33) | 1.67 | 0.26-10.64 | 0.589 |
| Grade | | | | | |
| Well/moderate differentiation (reference) | 26 | 8 (31) | 1.00 | | |
| Poor differentiation | 19 | 3 (16) | 0.42 | 0.10-1.87 | 0.256 |
| Tumor size (cm, continuous) | 45 | | 1.10 | 0.84-1.44 | 0.488 |
| Preoperative chemotherapy | | | | | |
| No (reference) | 37 | 9 (24) | 1.00 | | |
| Yes | 8 | 2 (25) | 1.04 | 0.18-6.07 | 0.968 |
| Preoperative radiation | | | | | |
| No (reference) | 41 | 10 (24) | 1.00 | | |
| Yes | 4 | 1 (25) | 1.03 | 0.10-11.08 | 0.978 |
| Smoking | | | | | |
| Never (reference) | 12 | 2 (17) | 1.00 | | |
| Former or current | 33 | 9 (27) | 1.87 | 0.34-10.27 | 0.469 |
| Multivariable logistic regression | | | | | |
| Sex | | | | | |
| Female (reference) | 27 | 4 (15) | 1.00 | | |
| Male | 18 | 7 (39) | 4.77 | 1.00-22.86 | 0.051 |
| Histologic subtype | | | | | 0.147 |
| Adenocarcinoma (reference) | 24 | 6 (25) | 1.00 | | |
| Squamous cell carcinoma | 10 | 5 (50) | 3.14 | 0.54-18.37 | 0.204 |
| Others* | 11 | 0 (0) | 0.25 | 0.02-2.50 | 0.236 |

*Neuroendocrine cancer and small cell lung cancer.

## Discussion

Inspired by our recent finding that GSR deficiency sensitized cancer cells to thioredoxin reductase inhibitors, we aimed to detect *GSR* deletions in primary lung cancers using FISH probes for *GSR* and the chromosome 8 centromere. Our results showed that *GSR* copy number loss

**Table 3.** Univariate regression analysis on aneuploidy and clinical parameters

| Parameter | No. of cases | Aneuploidy No. (%) | Hazard ratio | 95% confidence interval | *P* |
|---|---|---|---|---|---|
| Univariate logistic regression | | | | | |
| Sex | | | | | |
| Female (reference) | 27 | 9 (33) | 1.00 | | |
| Male | 18 | 10 (56) | 2.50 | 0.73-8.52 | 0.143 |
| Age in years (continuous) | 45 | | 1.05 | 0.99-1.13 | 0.124 |
| Histologic subtype | | | | | 0.766 |
| Adenocarcinoma (reference) | 24 | 9 (38) | 1.00 | | |
| Squamous cell carcinoma | 10 | 5 (50) | 1.67 | 0.41-6.77 | 0.717 |
| Others* | 11 | 5 (45) | 1.33 | 0.28-6.30 | 0.475 |
| Pathologic T category | | | | | 0.440 |
| T1 (reference) | 15 | 5 (33) | 1.00 | | |
| T2 | 21 | 11 (52) | 2.20 | 0.56-8.69 | 0.260 |
| T3-T4 | 9 | 3 (33) | 1.00 | 0.17-5.77 | 1.000 |
| Pathologic N category | | | | | |
| N0 (reference) | 28 | 13 (46) | 1.00 | | |
| N1-N2 | 17 | 6 (35) | 0.63 | 0.18-2.18 | 0.465 |
| Pathologic M category | | | | | |
| M0 (reference) | 39 | 17 (44) | 1.00 | | |
| M1 | 6 | 2 (33) | 0.65 | 0.11-3.96 | 0.638 |
| Grade | | | | | |
| Well/moderate differentiation (reference) | 26 | 10 (38) | 1.00 | | |
| Poor differentiation | 19 | 9 (47) | 1.44 | 0.43-4.77 | 0.551 |
| Tumor size (cm, continuous) | 45 | | 1.12 | 0.87-1.44 | 0.387 |
| Preoperative chemotherapy | | | | | |
| No (reference) | 37 | 16 (43) | 1.00 | | |
| Yes | 8 | 3 (38) | 0.79 | 0.16-3.79 | 0.766 |
| Preoperative radiation | | | | | |
| No (reference) | 41 | 16 (39) | 1.00 | | |
| Yes | 4 | 3 (75) | 4.69 | 0.45-49.08 | 0.197 |
| Cigarette smoking | | | | | |
| Never (reference) | 12 | 3 (25) | 1.00 | | |
| Former or current | 33 | 16 (48) | 2.82 | 0.65-12.33 | 0.168 |
| Multivariable logistic regression | | | | | |
| Preoperative radiation | | | | | |
| No (reference) | 41 | 16 (39) | 1.00 | | |
| Yes | 4 | 3 (75) | 18.63 | 0.90-384.17 | 0.058 |
| Cigarette smoking | | | | | |
| Never (reference) | 12 | 3 (25) | 1.00 | | |
| Former or current | 33 | 16 (48) | 7.59 | 0.86-66.75 | 0.068 |

*Neuroendocrine cancer and small cell lung cancer.

is common in primary lung adenocarcinoma and squamous cell carcinoma, and that chromosome 8 aneuploidy is common all histologic subtypes of lung cancer analyzed.

Aneuploidy, one of the hallmarks of cancer, can be caused by malfunctions in multiple molecular pathways that lead to chromosomal missegregation, including dysregulation of the spindle

assembly checkpoint [23, 24], aberrant microtubule kinetochore interactions [25], and abnormal centrosome separation [26]. Evidence has shown that aneuploidy acts as both a driver and a suppressor of tumorigenesis [27]. A meta-analysis of aneuploidy in non-small cell lung cancer based on cellular DNA content (DNA Index) assays showed that the overall frequency of aneuploidy was about 65% (ranging from 20% to 85%) and that aneuploidy was associated with poor overall survival [28]. Although aneuploidy in cancer cells is associated with multidrug resistance [29], aneuploid cells were found to be highly sensitive to some targeted agents, including compounds inducing energy stress or protein misfolding [30] and inhibitors of spindle assembly checkpoint kinase [31]. These findings suggest that aneuploidy may serve as a predictive biomarker for identification of responders to these agents. Moreover, aneuploid tumors were reported to have reduced levels of immune cell infiltration, particularly CD8+ lymphocyte infiltration, and weaker response to immunotherapy [32, 33], suggesting that aneuploidy may be involved in the response to cancer immunotherapy.

Using FISH analysis with probes for *GSR* and the chromosome 8 centromere, we found that 42% of primary lung cancer samples had chromosome 8 aneuploidy, and aneuploidy was detected in all histologic types analyzed. Univariate and multivariable logistic regression analysis with demographic and clinical variables showed that patients who had undergone preoperative radiation therapy or who had a self-reported history of cigarette smoking had an increased risk of aneuploidy, although these relationships did not reach statistical significance. Both cigarette smoking and radiation exposure have been reported to induce aneuploidy. Cigarette smoking increased the risk of aneuploidy in oral epithelial cells [34, 35] and human sperm [36], and proton and X-ray irradiation induced aneuploidy in human fibroblasts and cancer cells [37, 38].

Our results showed that *GSR* deletion was detectable in primary lung adenocarcinoma and squamous cell carcinoma at much higher frequencies than the deep deletions of *GSR* reported in The Cancer Genome Atlas database for these 2 diseases. One possible explanation for the high frequencies of *GSR* deletion detected by FISH is that FISH analysis, which provides information on *GSR* copy number changes in individual cells, is more sensitive than next-generation sequencing of the whole exome or whole genome, which provides information on gene copy number in tumor masses consisting of tumor cells and stromal cells. In fact, a previous study of microdissected primary lung tumors for loss of heterozygosity at 8p21-23 detected allelic losses at this region in 86% of small cell lung cancer specimens, 100% of squamous cell carcinoma specimens, and 81% of adenocarcinoma specimens [4]. These findings indicate that loss of chromosome arm 8p is common in primary lung cancers.

In the current study, we did not detect *GSR* deletion in our 3 small cell lung cancer specimens and 8 neuroendocrine cancer specimens, possibly because of the small number of specimens analyzed. Also, as shown in Figure S1, some patients with deletions in 8p21-23 genes, such as *TNFRSF10A*, *TNFRSF10G*, *DLC1*, and *GATA4*, did not have *GSR* deletion. Our results showed that male patients had a higher risk of *GSR* deletion than did female patients, although the *p* value was only 0.051 in multivariable analysis. The significance of this sex difference is therefore not clear. Nevertheless, in The Cancer Genome Atlas data, the frequency of *GSR* deletion or deletions of 8p21-23 genes was highest in prostate cancer (Figure S3). Whether sex is one of the factors contributing to chromosome arm 8p deletions in cancer remains to be further investigated. Because of critical roles of GSH in cancer metabolism and in response to anticancer therapy, *GSR* deletion as a biomarker for personalized therapy warrants further investigations.

**Acknowledgements**

This work was supported in part by the National Institutes of Health through grants R01CA-190628, Specialized Program of Research Excellence (SPORE) grant CA070907, The University of Texas PDX Development and Trial Center grant U54CA224065, and Cancer Center Support Grant P30CA016672; and by funds from the University Cancer Foundation via the Sister Institution Network Fund and the Lung Cancer Moon Shot Program at The University of Texas MD Anderson Cancer Center. We would like to thank Erica A Goodoff, Amy

Ninetto and the Department of Scientific Publications at The University of Texas MD Anderson Cancer Center for editorial review of the manuscript.

Fresh lung cancer samples were from surgically resected specimens under research protocols approved by the Institutional Review Board at The University of Texas MD Anderson Cancer Center. All clinical samples were collected with informed consent from the patients.

### Disclosure of conflict of interest

None.

**Address correspondence to:** Dr. Bingliang Fang, Department of Thoracic and Cardiovascular Surgery, The University of Texas MD Anderson Cancer Center, Unit 1515 Holcombe Blvd, 1489, Houston, TX 77030, USA. Tel: 713-563-9147; Fax: 713-794-4901; E-mail: bfang@mdanderson.org

### References

[1] Emi M, Fujiwara Y, Nakajima T, Tsuchiya E, Tsuda H, Hirohashi S, Maeda Y, Tsuruta K, Miyaki M and Nakamura Y. Frequent loss of heterozygosity for loci on chromosome 8p in hepatocellular carcinoma, colorectal cancer, and lung cancer. Cancer Res 1992; 52: 5368-72.

[2] Cai Y, Crowther J, Pastor T, Abbasi AL, Baietti MF, De TM, Vazquez I, Talebi A, Renzi F, Dehairs J, Swinnen JV and Sablina AA. Loss of chromosome 8p governs tumor progression and drug response by altering lipid metabolism. Cancer Cell 2016; 29: 751-766.

[3] Bethel CR, Faith D, Li X, Guan B, Hicks JL, Lan F, Jenkins RB, Bieberich CJ and De Marzo AM. Decreased NKX3.1 protein expression in focal prostatic atrophy, prostatic intraepithelial neoplasia, and adenocarcinoma: association with gleason score and chromosome 8p deletion. Cancer Res 2006; 66: 10683-90.

[4] Wistuba II, Behrens C, Virmani AK, Milchgrub S, Syed S, Lam S, Mackay B, Minna JD and Gazdar AF. Allelic losses at chromosome 8p21-23 are early and frequent events in the pathogenesis of lung cancer. Cancer Res 1999; 59: 1973-9.

[5] Weir BA, Woo MS, Getz G, Perner S, Ding L, Beroukhim R, Lin WM, Province MA, Kraja A, Johnson LA, Shah K, Sato M, Thomas RK, Barletta JA, Borecki IB, Broderick S, Chang AC, Chiang DY, Chirieac LR, Cho J, Fujii Y, Gazdar AF, Giordano T, Greulich H, Hanna M, Johnson BE, Kris MG, Lash A, Lin L, Lindeman N, Mardis ER, McPherson JD, Minna JD, Morgan MB, Nadel M, Orringer MB, Osborne JR, Ozenberger B, Ramos AH, Robinson J, Roth JA, Rusch V, Sasaki H, Shepherd F, Sougnez C, Spitz MR, Tsao MS, Twomey D, Verhaak RG, Weinstock GM, Wheeler DA, Winckler W, Yoshizawa A, Yu S, Zakowski MF, Zhang Q, Beer DG, Wistuba II, Watson MA, Garraway LA, Ladanyi M, Travis WD, Pao W, Rubin MA, Gabriel SB, Gibbs RA, Varmus HE, Wilson RK, Lander ES and Meyerson M. Characterizing the cancer genome in lung adenocarcinoma. Nature 2007; 450: 893-898.

[6] Kwek SS, Roy R, Zhou H, Climent J, Martinez-Climent JA, Fridlyand J and Albertson DG. Co-amplified genes at 8p12 and 11q13 in breast tumors cooperate with two major pathways in oncogenesis. Oncogene 2009; 28: 1892-1903.

[7] Kang J. Genomic alterations on 8p21-p23 are the most frequent genetic events in stage I squamous cell carcinoma of the lung. Exp Ther Med 2015; 9: 345-350.

[8] Moelans CB, van Maldegem CMG, van der Wall E and van Diest PJ. Copy number changes at 8p11-12 predict adverse clinical outcome and chemo- and radiotherapy response in breast cancer. Oncotarget 2018; 9: 17078-17092.

[9] Lebok P, Mittenzwei A, Kluth M, Ozden C, Taskin B, Hussein K, Moller K, Hartmann A, Lebeau A, Witzel I, Mahner S, Wolber L, Janicke F, Geist S, Paluchowski P, Wilke C, Heilenkotter U, Simon R, Sauter G, Terracciano L, Krech R, von der AA, Muller V and Burandt E. 8p deletion is strongly linked to poor prognosis in breast cancer. Cancer Biol Ther 2015; 16: 1080-7.

[10] Chua YL, Ito Y, Pole JC, Newman S, Chin SF, Stein RC, Ellis IO, Caldas C, O'Hare MJ, Murrell A and Edwards PA. The NRG1 gene is frequently silenced by methylation in breast cancers and is a strong candidate for the 8p tumour suppressor gene. Oncogene 2009; 28: 4041-4052.

[11] Xue W, Krasnitz A, Lucito R, Sordella R, Vanaelst L, Cordon-Cardo C, Singer S, Kuehnel F, Wigler M, Powers S, Zender L and Lowe SW. DLC1 is a chromosome 8p tumor suppressor whose loss promotes hepatocellular carcinoma. Genes Dev 2008; 22: 1439-1444.

[12] Enane FO, Shuen WH, Gu X, Quteba E, Przychodzen B, Makishima H, Bodo J, Ng J, Chee CL, Ba R, Seng Koh L, Lim J, Cheong R, Teo M, Hu Z, Ng KP, Maciejewski J, Radivoyevitch T, Chung A, Ooi LL, Tan YM, Cheow PC, Chow P, Chan CY, Lim KH, Yerian L, Hsi E, Toh HC and Saunthararajah Y. GATA4 loss of function in liver cancer impedes precur-

sor to hepatocyte transition. J Clin Invest 2017; 127: 3527-3542.
[13] Fisher MJ, Virmani AK, Wu L, Aplenc R, Harper JC, Powell SM, Rebbeck TR, Sidransky D, Gazdar AF and El-Deiry WS. Nucleotide substitution in the ectodomain of trail receptor DR4 is associated with lung cancer and head and neck cancer. Clin Cancer Res 2001; 7: 1688-97.
[14] Rubio-Moscardo F, Blesa D, Mestre C, Siebert R, Balasas T, Benito A, Rosenwald A, Climent J, Martinez JI, Schilhabel M, Karran EL, Gesk S, Esteller M, deLeeuw R, Staudt LM, Fernandez-Luna JL, Pinkel D, Dyer MJ and Martinez-Climent JA. Characterization of 8p21.3 chromosomal deletions in B-cell lymphoma: TRAIL-R1 and TRAIL-R2 as candidate dosage-dependent tumor suppressor genes. Blood 2005; 106: 3214-3222.
[15] Couto N, Wood J and Barber J. The role of glutathione reductase and related enzymes on cellular redox homoeostasis network. Free Radic Biol Med 2016; 95: 27-42.
[16] Circu ML and Aw TY. Glutathione and modulation of cell apoptosis. Biochim Biophys Acta 2012; 1823: 1767-1777.
[17] Gordillo GM, Biswas A, Khanna S, Spieldenner JM, Pan X and Sen CK. Multidrug resistance-associated protein-1 (MRP-1)-dependent glutathione Disulfide (GSSG) efflux as a critical survival factor for oxidant-enriched tumorigenic endothelial cells. J Biol Chem 2016; 291: 10089-10103.
[18] Benhar M, Shytaj IL, Stamler JS and Savarino A. Dual targeting of the thioredoxin and glutathione systems in cancer and HIV. J Clin Invest 2016; 126: 1630-1639.
[19] Bansal A and Simon MC. Glutathione metabolism in cancer progression and treatment resistance. J Cell Biol 2018; 217: 2291-2298.
[20] Zhu Z, Du S, Du Y, Ren J, Ying G and Yan Z. Glutathione reductase mediates drug resistance in glioblastoma cells by regulating redox homeostasis. J Neurochem 2018; 144: 93-104.
[21] Kim SJ, Jung HJ, Hyun DH, Park EH, Kim YM and Lim CJ. Glutathione reductase plays an anti-apoptotic role against oxidative stress in human hepatoma cells. Biochimie 2010; 92: 927-932.
[22] Yan X, Zhang X, Wang L, Zhang R, Pu X, Wu S, Li L, Tong P, Wang J, Meng Q, Jensen VB, Girard L, Minna JD, Roth JA, Swisher SG, Heymach JV and Fang B. Inhibition of thioredoxin/thioredoxin reductase induces synthetic lethality in lung cancers with compromised glutathione homeostasis. Cancer Res 2019; 79: 25-132.
[23] Hanks S, Coleman K, Reid S, Plaja A, Firth H, Fitzpatrick D, Kidd A, Mehes K, Nash R, Robin N, Shannon N, Tolmie J, Swansbury J, Irrthum A, Douglas J and Rahman N. Constitutional aneuploidy and cancer predisposition caused by biallelic mutations in BUB1B. Nat Genet 2004; 36: 1159-1161.
[24] Sotillo R, Hernando E, Diaz-Rodriguez E, Teruya-Feldstein J, Cordon-Cardo C, Lowe SW and Benezra R. Mad2 overexpression promotes aneuploidy and tumorigenesis in mice. Cancer Cell 2007; 11: 9-23.
[25] Cimini D, Howell B, Maddox P, Khodjakov A, Degrassi F and Salmon ED. Merotelic kinetochore orientation is a major mechanism of aneuploidy in mitotic mammalian tissue cells. J Cell Biol 2001; 153: 517-527.
[26] Nam HJ and van Deursen JM. Cyclin B2 and p53 control proper timing of centrosome separation. Nat Cell Biol 2014; 16: 538-549.
[27] Weaver BA, Silk AD, Montagna C, Verdier-Pinard P and Cleveland DW. Aneuploidy acts both oncogenically and as a tumor suppressor. Cancer Cell 2007; 11: 25-36.
[28] Choma D, Daures JP, Quantin X and Pujol JL. Aneuploidy and prognosis of non-small-cell lung cancer: a meta-analysis of published data. Br J Cancer 2001; 85: 14-22.
[29] Duesberg P, Stindl R and Hehlmann R. Origin of multidrug resistance in cells with and without multidrug resistance genes: chromosome reassortments catalyzed by aneuploidy. Proc Natl Acad Sci U S A 2001; 98: 11283-11288.
[30] Tang YC, Williams BR, Siegel JJ and Amon A. Identification of aneuploidy-selective antiproliferation compounds. Cell 2011; 144: 499-512.
[31] Libouban MAA, de Roos JADM, Uitdehaag JCM, Willemsen-Seegers N, Mainardi S, Dylus J, de Man J, Tops B, Meijerink JPP, Storchová Z, Buijsman RC, Medema RH, Zaman GJR. Stable aneuploid tumors cells are more sensitive to TTK inhibition than chromosomally unstable cell lines. Oncotarget 2017; 8: 38309-38325.
[32] Davoli T, Uno H, Wooten EC and Elledge SJ. Tumor aneuploidy correlates with markers of immune evasion and with reduced response to immunotherapy. Science 2017; 355.
[33] Taylor AM, Shih J, Ha G, Gao GF, Zhang X, Berger AC, Schumacher SE, Wang C, Hu H, Liu J, Lazar AJ, Cherniack AD, Beroukhim R and Meyerson M. Genomic and functional approaches to understanding cancer aneuploidy. Cancer Cell 2018; 33: 676-689, e673.
[34] Souto GR, Caliari MV, Lins CE, de Aguiar MC, de Abreu MH and Mesquita RA. Tobacco use increase the number of aneuploid nuclei in the clinically healthy oral epithelium. J Oral Pathol Med 2010; 39: 605-610.
[35] Sudbo J, Samuelsson R, Risberg B, Heistein S, Nyhus C, Samuelsson M, Puntervold R, Sigstad E, Davidson B, Reith A and Berner A. Risk

markers of oral cancer in clinically normal mucosa as an aid in smoking cessation counseling. J Clin Oncol 2005; 23: 1927-1933.

[36] Shi Q, Ko E, Barclay L, Hoang T, Rademaker A and Martin R. Cigarette smoking and aneuploidy in human sperm. Mol Reprod Dev 2001; 59: 417-421.

[37] Sgura A, Antoccia A, Cherubini R and Tanzarella C. Chromosome nondisjunction and loss induced by protons and X rays in primary human fibroblasts: role of centromeres in aneuploidy. Radiat Res 2001; 156: 225-231.

[38] Park MT, Oh ET, Song MJ, Lee H, Choi EK and Park HJ. NQO1 prevents radiation-induced aneuploidy by interacting with Aurora-A. Carcinogenesis 2013; 34: 2470-2485.

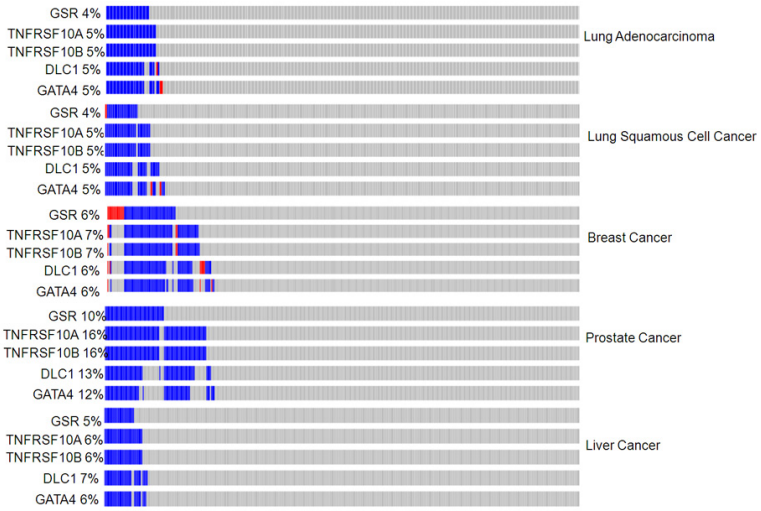


**Figure S1.** Alterations of *GSR* and 8p21-23 genes identified in The Cancer Genome Atlas database. Genes and the percentage of samples showing changes are indicated on the left. Blue: deep deletions, red: Amplification. The data were obtained from cbioportal.org

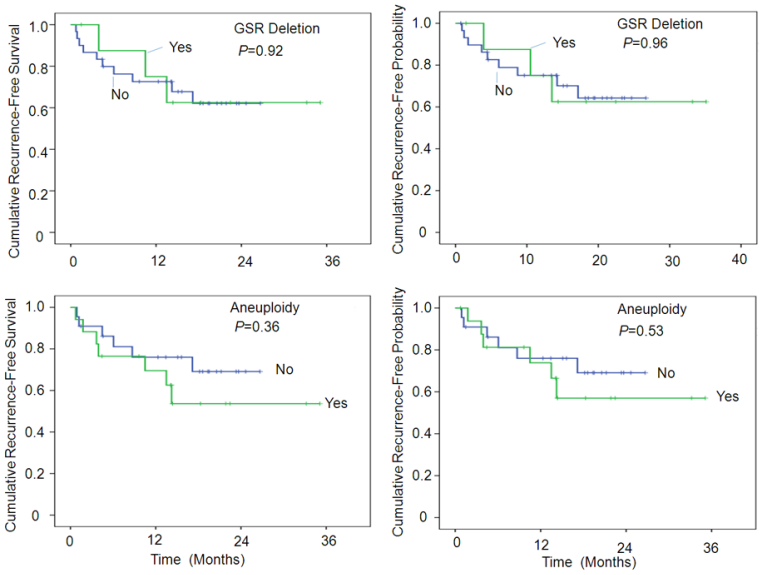


**Figure S2.** Kaplan-Meier curves comparing disease-free survival and probability of recurrence in lung cancer patients with or without GSR deletion or aneuploidy.

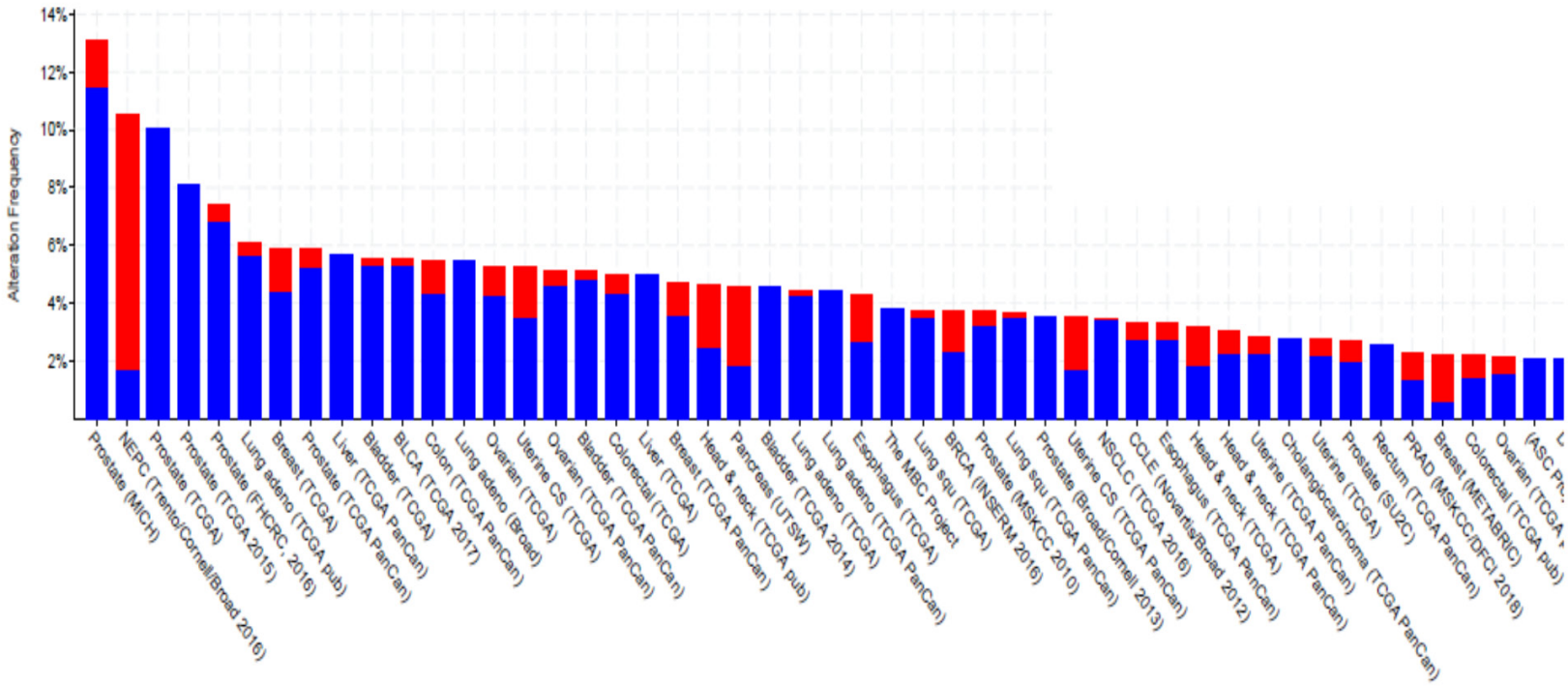


**Figure S3.** GSR copy number alterations in different types of cancers. The data were retrieved from cbioportal.org by searching GSR for copy number alterations. The frequency of GSR deletion was highest in prostate cancer.