



Oxidative Stress, Nrf2, and Epigenetic Modification Contribute to Anticancer Drug Resistance

Kyoung Ah Kang and Jin Won Hyun

Department of Biochemistry, School of Medicine, Jeju National University, Jeju, Korea

(Received September 19, 2016; Revised November 24, 2016; Accepted December 2, 2016)

Nuclear factor E2-related factor 2 (Nrf2), a transcription factor, controls the expression of genes encoding cytoprotective proteins, including antioxidant enzymes that combat oxidative and electrophilic stress to maintain redox homeostasis. However, recent studies demonstrated that, in cancer, aberrant activation of Nrf2 by epigenetic alterations promotes high expression of cytoprotective proteins, which can decrease the efficacy of anticancer drugs used for chemotherapy. In this review, we summarize recent findings regarding the relationship between oxidative stress, Nrf2, epigenetic modification, and anticancer drug resistance, which should aid in development of new strategies to improve chemotherapeutic efficacy.

Key words: Anticancer drug resistance, Oxidative stress, Nrf2 transcription factor, Epigenetic modification

INTRODUCTION

Nuclear factor E2-related factor 2 (Nrf2), a transcription factor that regulates multiple antioxidant and detoxifying enzymes, is primarily involved in adaptation to various cellular stresses. High Nrf2 activity makes a critical contribution to cancer cell resistance to various anticancer therapies (1,2). Epigenetic modifications, which are heritable alterations in gene expression not accompanied by changes in the primary DNA sequence, are intimately involved in oxidative stress and Nrf2 expression (3). In this review, we discuss the latest findings regarding epigenetic regulation of Nrf2 expression by DNA demethylation and histone methylation in 5-fluorouracil-resistant cells. Studies of the epigenetic modification in Nrf2 expression will provide new candidate therapeutic targets for anticancer drug resistance.

ANTICANCER DRUG RESISTANCE AND OXIDATIVE STRESS

Anticancer drugs rely primarily on induction of cell death via apoptosis, mitotic catastrophe, or premature senescence.

Correspondence to: Jin Won Hyun, Department of Biochemistry, School of Medicine, Jeju National University, 102 Jejudaehakro, Jeju 63243, Korea
E-mail: jinwonh@jejunu.ac.kr

This is an Open-Access article distributed under the terms of the Creative Commons Attribution Non-Commercial License (<http://creativecommons.org/licenses/by-nc/3.0>) which permits unrestricted non-commercial use, distribution, and reproduction in any medium, provided the original work is properly cited.

However, anticancer drug resistance and dose-limiting toxicities are major obstacles to achieving successful cancer chemotherapy (4). The mechanisms underlying the development of anticancer drug resistance are complex and often involve activation of antioxidant or detoxifying enzymes (5,6). Studies of anticancer drug resistance have yielded valuable information about how to improve cancer chemotherapy by circumventing such resistance.

Reactive oxygen species (ROS) comprise hydrogen peroxide (H₂O₂), hydroxyl radical ([•]OH), superoxide (O₂⁻), and singlet oxygen (¹O₂). Under physiological conditions, cells can maintain a balance between cellular oxidants and antioxidants, a state referred to as redox homeostasis. Many types of tumor cells contain higher levels of ROS than normal cells, promoting cancer progression and development (7). On the other hand, when ROS concentrations become extremely high, they cause tumor cell death (8). Thus, a variety of drugs with direct or indirect effects on ROS levels have been employed as effective cancer therapies. In certain cancer cells, persistent oxidative stress may induce adaptive stress responses, including activation of redox-sensitive transcription factors such as Nrf2, leading to an increase in the expression of ROS-scavenging enzymes, elevated levels of survival factors, and suppression of cell death factors. ROS-mediated DNA mutations or deletions promote genomic instability, providing an additional mechanism for stress adaptation. Together, these events enable tumor cells to maintain cellular viability with high levels of ROS. Because these transcription factors also regulate the expression of genes that are responsible for proliferation, evasion of senescence, angiogenesis, and metastasis, redox

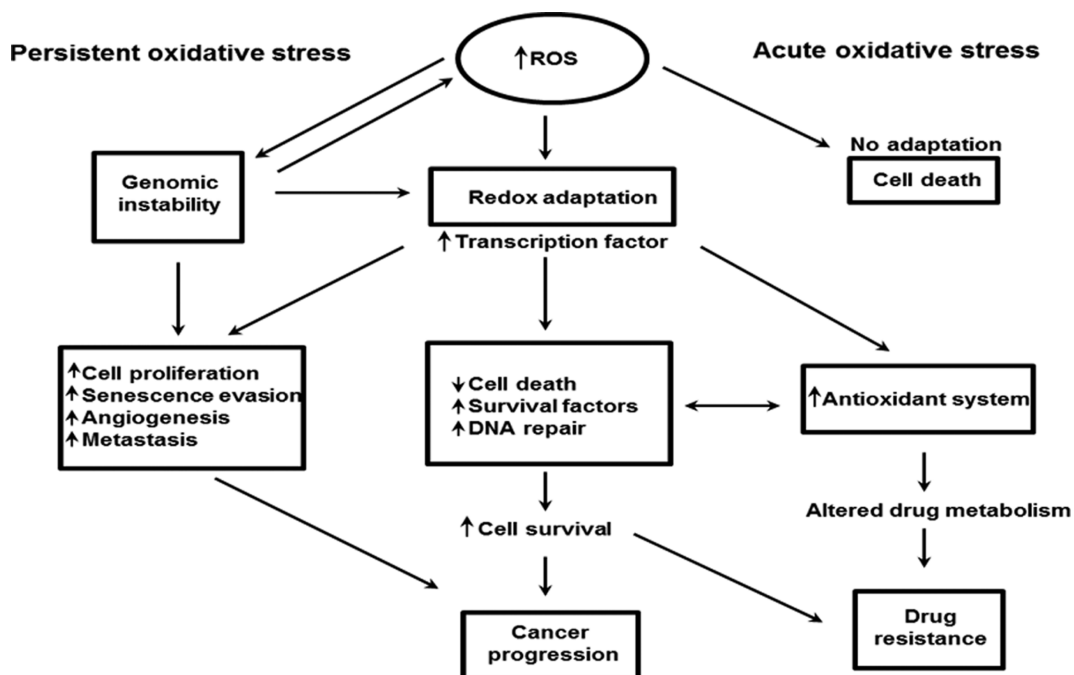


Fig. 1. Oxidative stress status in anticancer drug resistance (8). Drug-induced persistent oxidative stress can be overcome by increasing genomic instability and by redox adaptation. Both processes are implicated in cancer progression and drug resistance.

adaptation may promote cancer development. The resultant alterations in drug metabolism, together with elevated cell survival, may render cancer cells more resistant to chemotherapeutic agents (8-10) (Fig. 1). Accordingly, manipulating ROS levels via redox modulation represents a promising strategy for selectively killing cancer cells without causing significant toxicity in normal cells.

NUCLEAR FACTOR E2-RELATED FACTOR 2 (Nrf2)

The transcription factor Nrf2 binds to small Maf proteins at the antioxidant response element (ARE) in the promoter regions of target genes, and Kelch-like ECH-associated protein 1 (Keap1), a cytoplasmic anchor of Nrf2, promotes Nrf2 degradation by the ubiquitin proteasome pathway (11, 12). The target genes for Nrf2 are multiple antioxidant enzymes, including heme oxygenase 1 (HO-1), catalase, superoxide dismutase, NAD(P)H quinone oxidoreductase 1, glutamate-cysteine ligase, glutathione S-transferase, and glutathione synthetase.

Several studies reported that suppression of carcinogenesis by chemopreventive drugs is mediated through activation of Nrf2 (13,14). Paradoxically, however, recent studies showed that oncogene-induced Nrf2 promotes both ROS detoxification and tumorigenesis (12,15). Moreover, genetic analyses of human cancers revealed that Nrf2 may be oncogenic and cause resistance to chemotherapy (16). Na and Surh reviewed the evidence that the cellular stress response or cytoprotective signaling mediated via the Nrf2-HO-1

axis is exploited by cancer cells to promote their proliferation and increase their ability to survive chemotherapy (17). Therefore, Nrf2 and its downstream target HO-1 represent candidate therapeutic targets for the management of cancer.

EPIGENETIC MODIFICATIONS

Epigenetics is the molecular phenomenon by which phenotypic changes are transmitted from one generation to another with no accompanying alterations in the DNA sequence. Epigenetic mechanisms include DNA methylation/demethylation, histone modifications, and microRNA (miRNA)-mediated regulation. Although many types of histone modifications have been reported, here we focus on methylation/demethylation of DNA and histones.

DNA methylation/demethylation. DNA methylation occurs when methyl groups are incorporated into cytosine molecules by DNA methyltransferases (DNMTs), forming 5-methylcytosine (5-mC). In particular, two *de novo* DNMTs (DNMT3A, DNMT3B) and a maintenance DNMT (DNMT1) transfer methyl groups from S-adenosyl-methionine to cytosine in CpG dinucleotides. This process contributes to the suppression of transcription. Methylation can be reversed by DNA demethylases, the ten-eleven translocation enzymes (TETs): TET1, TET2, and TET3. These enzymes convert 5-mC to 5-hydroxymethylcytosine (5-hmC), 5-formylcytosine (5-fC), and 5-carboxylcytosine (5-caC), which are subsequently excised from the DNA by

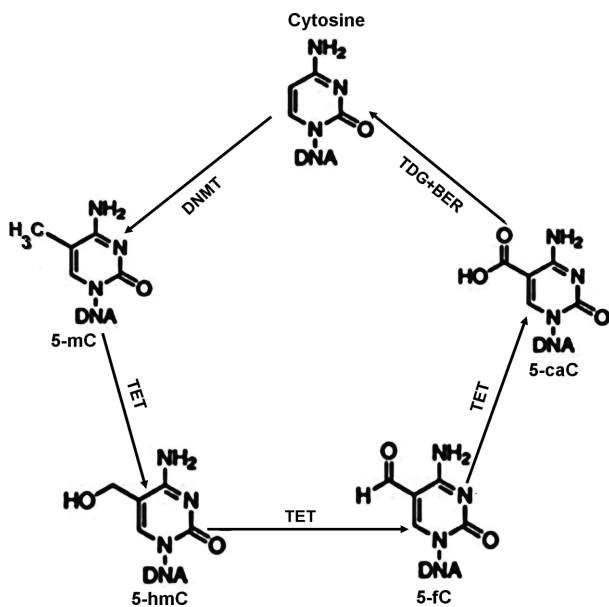


Fig. 2. Cytosine methylation and demethylation (18,19). DNMTs catalyze conversion of cytosine into 5-mC. TET proteins can iteratively oxidize 5-mC to 5-hmC, 5-fC, and 5-caC. Thymine DNA glycosylase (TDG) can decarboxylate either 5-fC or 5-caC and replace it with an unmodified cytosine through base excision repair (BER), completing the cycle of dynamic cytosine removal.

glycosylases (18,19) (Fig. 2). Generally, DNA demethylation contributes to the activation of transcription.

Histone methylation/demethylation. Histone methylation can either repress or activate transcription, depending on several factors: arginine methylation of histone H3 and H4 promotes transcriptional activation, whereas lysine methylation of histone H3 and H4 is implicated in both transcriptional activation and repression, depending on the methylation site. In addition, lysine residues can be mono-, di-, or tri-methylated, providing further functional diversity to each methylation site. Tri-methylation on K4 of histone H3 (H3K4Me₃) by the mixed lineage leukemia (MLL) protein is generally associated with transcriptional activation (20), whereas H3K9Me₂, H3K9Me₃, and H3K27Me₃ generated by G9a or enhancer of zeste homolog 2 (EZH2) are generally associated with transcriptional repression (21). Histone demethylases, e.g., the lysine-specific demethylase 1 and Jumonji domain-containing histone demethylases, are involved in demethylation of mono-, di-, and tri-methylated lysines (22).

RELATIONSHIP BETWEEN DRUG RESISTANCE, OXIDATIVE STRESS, Nrf2, AND EPIGENETIC MODIFICATIONS

The epigenetic regulation of Keap1 has been investigated in various cancers. Wang and colleagues first reported that

Keap1 is highly expressed in BEAS-2B human normal bronchial epithelial cells but is down-regulated in a series of lung cancer cell lines and human lung cancer tissues; this down-regulation was accompanied by the hypermethylation of CpG sites in the Keap1 promoter region and was restored by 5-aza treatment (23). Similar results have been obtained in various cancers, including malignant gliomas and breast, colorectal, prostate, thyroid, and head and neck cancer cells. Activation of Nrf2 signaling by hypermethylation of Keap1 promoter is associated with tumor progression and resistance to therapies (24). Li and colleagues showed that decreased EZH2 expression significantly correlated with elevated expression of Nrf2, NQO1, and HO-1 in lung cancer tissues and cell lines, which was mainly attributed to a decrease in H3K27Me₃ in the Nrf2 promoter (25). Recently, Kang and colleagues focused on the causative relationship between Nrf2 expression and epigenetic alterations, especially DNA methylation at cytosines during 5-fluorouracil (FU)-induced oxidative stress (26). They found that 5-FU-induced ROS activate DNA demethylases (TETs), resulting in hypomethylation of the Nrf2 promoter and activation of Nrf2 expression. This, in turn, upregulates expression of the antioxidant enzyme HO-1, a Nrf2 target, resulting in resistance to 5-FU. In addition, in mice implanted with shNrf2-transfected 5-FU-resistant SNUC5 (SNUC5/5-FUR) colon cancer cells, 5-FU treatment decreased tumor volume, size, and weight, and increased the proportion of apoptotic cells to a greater extent than in mice implanted with shControl-transfected SNUC5/5-FUR cells. Thus, high Nrf2 expression resulting from oxidative stress-induced DNA demethylation may promote resistance to 5-FU (26). Kang and colleagues demonstrated that, in addition to DNA methylation, histone methylation status under 5-FU-induced oxidative stress also influences expression of Nrf2 and its target antioxidant enzymes, resulting in resistance to 5-FU. Levels of MLL and the modification it catalyzes (H3K4Me₃) were higher in SNUC5/5-FUR cells than in SNUC5 cells, and siRNA knockdown of MLL in these cells significantly decreased expression of Nrf2 and HO-1 (27). Furthermore, Kang and colleagues demonstrated that the TETs and MLL interact with O-GlcNAc transferase (OGT) and host cell factor 1 (HCF1) to regulate Nrf2 expression (27). Several reports showed that the TETs-OGT interaction increases TET DNA demethylation activity and recruit histone methyltransferase complex required to a high H3K4Me₃ chromatin environment, resulting in transcriptional activation (28-30).

MLL is a component of the complex of proteins associated with Set1 (COMPASS) like complex. HCF1, a component of the MLL complex, is critical for the integrity of the MLL/COMPASS-like complex. Capotosti and colleagues suggested that HCF1 is GlcNAcylated and undergoes proteolytic maturation in the active site of OGT. This OGT link is associated with activation of the regulatory functions of

HCF1 (31). In addition, a previous study suggested that HCF1 functions as a transcriptional switch for the action of MLL (32). siRNA knockdown of HCF1 decreases the level of MLL mRNA and protein in SNUC5/5-FUR cells, resulting in reduced expression of Nrf2 protein (27). These results suggest that the MLL and HCF1 components of the MLL/COMPASS-like complex are necessary for Nrf2 expression.

Recent work showed that OGT stabilizes the MLL protein: depletion of OGT in cells decreased the level of MLL via ubiquitin/proteasome-dependent proteolytic degradation, whereas ectopic expression of OGT protein suppressed MLL ubiquitylation (33). siRNA-mediated knockdown of OGT decreased mRNA and protein levels of MLL in SNUC5/5-FUR cells, resulting in reduced expression of HCF1 and Nrf2 proteins (27). In addition, upregulation of Nrf2 by the TET1-OGT-MLL complex was confirmed by ChIP assay: the level of H3K4Me3 on the Nrf2 promoter was significantly elevated in SNUC5/5-FUR cells, whereas the H3K4Me3 level on the β -actin promoter, which is unre-

lated to 5-FU resistance, was unchanged (27). Thus, oxidative stress-activated TET1 demethylates DNA in the promoter of Nrf2. In addition, TET1 recruits OGT and the MLL/COMPASS-like complex to the promoter. The complex methylates H3K4, resulting in transcriptional activation of Nrf2, ultimately leading to chemo-resistance (Fig. 3).

CONCLUSION

A recent study elucidated the epigenetic regulation of Nrf2 expression in 5-FU-resistant cancer cells (26,27). As depicted schematically in Figure 3, Nrf2 expression can be activated by DNA demethylation in CpGs of promoter regions, as well as histone methylation. However, although oxidative stress-induced anticancer drug resistance is involved in these epigenetic modifications, it is not clear whether epigenetic processes other than DNA demethylation and histone methylation also contribute. The exact mechanisms of epigenetic regulation of Nrf2 expression in anticancer drug resistance, as well as its physiological significance, remain open for further investigation.

ACKNOWLEDGMENTS

This study was supported by a grant from the National R&D Program for Cancer Control, Ministry for Health and Welfare, Republic of Korea (1120340) and by the National Research Foundation of Korea Grant funded by the Korean Government (MEST) (NRF-2016R1A2B4007934).

REFERENCE

- Bai, X., Chen, Y., Hou, X., Huang, M. and Jin, J. (2016) Emerging role of NRF2 in chemoresistance by regulating drug-metabolizing enzymes and efflux transporters. *Drug Metab. Rev.*, **48**, 541-567.
- Furfaro, A.L., Piras, S., Domenicotti, C., Fenoglio, D., De Luigi, A., Salmona, M., Moretta, L., Marinari, U.M., Pronzato, M.A., Traverso, N. and Nitti, M. (2016) Role of Nrf2, HO-1 and GSH in neuroblastoma cell resistance to bortezomib. *PLoS ONE*, **11**, e0152465.
- Guo, Y., Yu, S., Zhang, C. and Kong, A.N. (2015) Epigenetic regulation of Keap1-Nrf2 signaling. *Free Radic. Biol. Med.*, **88**, 337-349.
- Seton-Rogers, S. (2016) Chemotherapy: preventing competitive release. *Nat. Rev. Cancer*, **16**, 199.
- Holohan, C., Van Schaeybroeck, S., Longley, D.B. and Johnston, P.G. (2013) Cancer drug resistance: an evolving paradigm. *Nat. Rev. Cancer*, **13**, 714-726.
- Ramos, P. and Bentires-Alj, M. (2015) Mechanism-based cancer therapy: resistance to therapy, therapy for resistance. *Oncogene*, **34**, 3617-3626.
- Gorrini, C., Harris, I.S. and Mak, T.W. (2013) Modulation of oxidative stress as an anticancer strategy. *Nat. Rev. Drug Discov.*, **12**, 931-947.
- Trachootham, D., Alexandre, J. and Huang, P. (2009) Target-

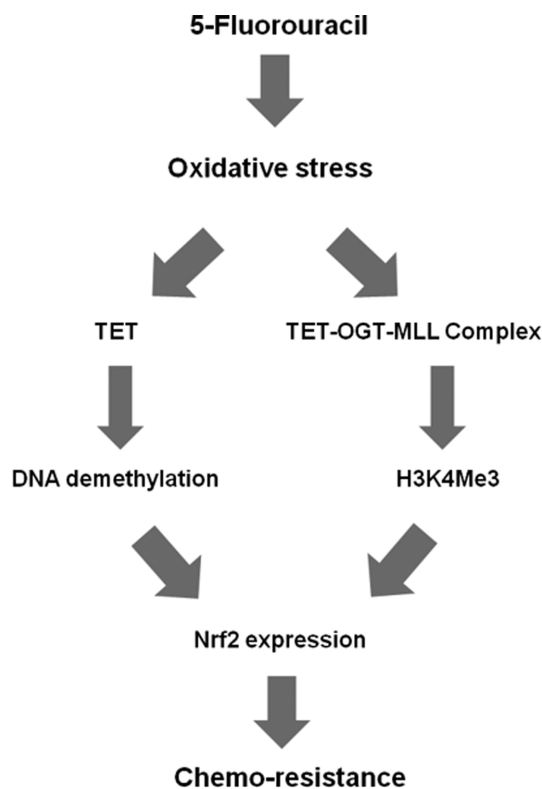


Fig. 3. Model: the roles of DNA demethylation and histone methylation in the mechanism of resistance to 5-FU (26,27). 5-FU induces oxidative stress via generation of ROS, which increase TET1 activity. Induction of TET1 leads to activation of Nrf2, ultimately causing drug resistance. Moreover, oxidative stress-activated TET1 recruits OGT to the Nrf2 promoter. OGT GlcNAcylates HCF1, a component of the MLL/COMPASS-like complex. This complex methylates H3K4, resulting in transcriptional activation of Nrf2, leading to chemo-resistance.

- ing cancer cells by ROS-mediated mechanisms: a radical therapeutic approach? *Nat. Rev. Drug Discov.*, **8**, 579-591.
9. Liu, Y., Li, Q., Zhou, L., Xie, N., Nice, E.C., Zhang, H., Huang, C. and Lei, Y. (2016) Cancer drug resistance: redox resetting renders a way. *Oncotarget*, **7**, 42740-42761.
 10. Debatin, K.M. and Kramer, P.H. (2004) Death receptors in chemotherapy and cancer. *Oncogene*, **23**, 2950-2966.
 11. Ren, D., Villeneuve, N.F., Jiang, T., Wu, T., Lau, A., Toppin, H.A. and Zhang, D.D. (2011) Brusatol enhances the efficacy of chemotherapy by inhibiting the Nrf2-mediated defense mechanism. *Proc. Natl. Acad. Sci. U.S.A.*, **108**, 1433-1438.
 12. DeNicola, G.M., Karreth, F.A., Humpton, T.J., Gopinathan, A., Wei, C., Frese, K., Mangal, D., Yu, K.H., Yeo, C.J., Calhoun, E.S., Scrimieri, F., Winter, J.M., Hruban, R.H., Iacobuzio-Donahue, C., Kern, S.E., Blair, I.A. and Tuveson, D.A. (2011) Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. *Nature*, **475**, 106-109.
 13. Zhang, Y. and Gordon, G.B. (2004) A strategy for cancer prevention: stimulation of the Nrf2-ARE signaling pathway. *Mol. Cancer Ther.*, **3**, 885-893.
 14. Giudice, A., Arra, C. and Turco, M.C. (2010) Review of molecular mechanisms involved in the activation of the Nrf2-ARE signaling pathway by chemopreventive agents. *Methods Mol. Biol.*, **647**, 37-74.
 15. Chio, I.I., Jafarnejad, S.M., Ponz-Sarvisé, M., Park, Y., Rivera, K., Palm, W., Wilson, J., Sangar, V., Hao, Y., Öhlund, D., Wright, K., Filippini, D., Lee, E.J., Da Silva, B., Schoepfer, C., Wilkinson, J.E., Buscaglia, J.M., DeNicola, G.M., Tiriác, H., Hammell, M., Crawford, H.C., Schmidt, E.E., Thompson, C.B., Pappin, D.J., Sonenberg, N. and Tuveson, D.A. (2016) NRF2 promotes tumor maintenance by modulating mRNA translation in pancreatic cancer. *Cell*, **166**, 963-976.
 16. Sporn, M.B. and Liby, K.T. (2012) NRF2 and cancer: the good, the bad and the importance of context. *Nat. Rev. Cancer*, **12**, 564-571.
 17. Na, H.K. and Surh, Y.J. (2014) Oncogenic potential of Nrf2 and its principal target protein heme oxygenase-1. *Free Radic. Biol. Med.*, **67**, 353-365.
 18. Shen, L., Song, C.X., He, C. and Zhang, Y. (2014) Mechanism and function of oxidative reversal of DNA and RNA methylation. *Annu. Rev. Biochem.*, **83**, 585-614.
 19. Ito, S., Shen, L., Dai, Q., Wu, S.C., Collins, L.B., Swenberg, J.A., He, C. and Zhang, Y. (2011) Tet proteins can convert 5-methylcytosine to 5-formylcytosine and 5-carboxylcytosine. *Science*, **333**, 1300-1303.
 20. Shilatifard, A. (2008) Molecular implementation and physiological roles for histone H3 lysine 4 (H3K4) methylation. *Curr. Opin. Cell Biol.*, **20**, 341-348.
 21. Coward, W.R., Feghali-Bostwick, C.A., Jenkins, G., Knox, A.J. and Pang, L. (2014) A central role for G9a and EZH2 in the epigenetic silencing of cyclooxygenase-2 in idiopathic pulmonary fibrosis. *FASEB J.*, **28**, 3183-3196.
 22. Yang, D., Okamura, H., Teramachi, J. and Haneji, T. (2016) Histone demethylase Jmjd3 regulates osteoblast apoptosis through targeting anti-apoptotic protein Bcl-2 and pro-apoptotic protein Bim. *Biochim. Biophys. Acta*, **1863**, 650-659.
 23. Wang, R., An, J., Ji, F., Jiao, H., Sun, H. and Zhou, D. (2008) Hypermethylation of the Keap1 gene in human lung cancer cell lines and lung cancer tissues. *Biochem. Biophys. Res. Commun.*, **373**, 151-154.
 24. Muscarella, L.A., Barbano, R., D'Angelo, V., Copetti, M., Cocco, M., Balsamo, T., la Torre, A., Notarangelo, A., Troiano, M., Parisi, S., Icolaro, N., Catapano, D., Valori, V. M., Pellegrini, F., Merla, G., Carella, M., Fazio, V.M. and Parrella, P. (2011) Regulation of Keap1 expression by promoter methylation in malignant gliomas and association with patient's outcome. *Epigenetics*, **6**, 317-325.
 25. Li, Z., Xu, L., Tang, N., Xu, Y., Ye, X., Shen, S., Niu, X., Lu, S. and Chen, Z. (2014) The polycomb group protein EZH2 inhibits lung cancer cell growth by repressing the transcription factor Nrf2. *FEBS Lett.*, **588**, 3000-3007.
 26. Kang, K.A., Piao, M.J., Kim, K.C., Kang, H.K., Chang, W.Y., Park, I.C., Keum, Y.S., Surh, Y.J. and Hyun, J.W. (2014) Epigenetic modification of Nrf2 in 5-fluorouracil-resistant colon cancer cells: involvement of TET-dependent DNA demethylation. *Cell Death Dis.*, **5**, e1183.
 27. Kang, K.A., Piao, M.J., Ryu, Y.S., Kang, H.K., Chang, W.Y., Keum, Y.S. and Hyun, J.W. (2016) Interaction of DNA demethylase and histone methyltransferase upregulates Nrf2 in 5-fluorouracil-resistant colon cancer cells. *Oncotarget*, **7**, 40594-40620.
 28. Mohan, M., Herz, H.M., Smith, E.R., Zhang, Y., Jackson, J., Washburn, M.P., Florens, L., Eisenberg, J.C. and Shilatifard, A. (2011) The COMPASS family of H3K4 methylases in Drosophila. *Mol. Cell. Biol.*, **31**, 4310-4318.
 29. Deplus, R., Delatte, B., Schwinn, M.K., Defrance, M., Méndez, J., Murphy, N., Dawson, M.A., Volkmar, M., Putmans, P., Calonne, E., Shih, A.H., Levine, R.L., Bernard, O., Mercher, T., Solary, E., Urh, M., Daniels, D.L. and Fuks, F. (2013) TET2 and TET3 regulate GlcNAcylation and H3K4 methylation through OGT and SET1/COMPASS. *EMBO J.*, **32**, 645-655.
 30. Ardehali, M.B., Mei, A., Zobeck, K.L., Caron, M., Lis, J.T. and Kusch, T. (2011) Drosophila Set1 is the major histone H3 lysine 4 trimethyltransferase with role in transcription. *EMBO J.*, **30**, 2817-2828.
 31. Capotosti, F., Guernier, S., Lammers, F., Waridel, P., Cai, Y., Jin, J., Conaway, J.W., Conaway, R.C. and Herr, W. (2011) O-GlcNAc transferase catalyzes site-specific proteolysis of HCF-1. *Cell*, **144**, 376-388.
 32. Yokoyama, A., Wang, Z., Wysocka, J., Sanyal, M., Aufiero, D.J., Kitabayashi, I., Herr, W. and Cleary, M.L. (2004) Leukemia proto-oncoprotein MLL forms a SET1-like histone methyltransferase complex with menin to regulate Hox gene expression. *Mol. Cell. Biol.*, **24**, 5639-5649.
 33. Ding, X., Jiang, W., Zhou, P., Liu, L., Wan, X., Yuan, X., Wang, X., Chen, M., Chen, J., Yang, J., Kong, C., Li, B., Peng, C., Wong, C.C., Hou, F. and Zhang, Y. (2015) Mixed lineage leukemia 5 (MLL5) protein stability is cooperatively regulated by O-GlcNAc transferase (OGT) and ubiquitin specific protease 7 (USP7). *PLoS ONE*, **10**, e0145023.