Fundamental Toxicological Sciences (Fundam. Toxicol. Sci.)
Vol.3, No.3, 137-142, 2016

Original Article

GCN5-deficiency remarkably enhances the sensitivity of B cells in response to 4-nitroquinoline 1-oxide

Hidehiko Kikuchi[1,2], Futoshi Kuribayashi[2,3], Hitomi Mimuro[2] and Shinobu Imajoh-Ohmi[4]

[1]Laboratory of Biological Chemistry, Department of Food and Nutrition, Shokei University Junior College, 2-6-78 Kuhonji, Chuo-ku, Kumamoto 862-8678, Japan
[2]Division of Bacteriology, Department of Infectious Diseases Control, International Research Center for Infectious Diseases, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, 108-8639, Japan
[3]Department of Biochemistry, Kawasaki Medical School, 577 Matsushima, Kurashiki, Okayama, 701-0192, Japan
[4]Laboratory Center for Proteomics Research, Graduate School of Frontier Sciences, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Tokyo, 108-8639, Japan

(Received April 23, 2016; Accepted May 4, 2016)

ABSTRACT — A typical DNA-damaging agent 4-nitroquinoline 1-oxide (4NQO) is known as an experimental oral carcinogen. Although 4NQO was initially characterized as a UV-mimetic agent, it shows more complex effects inducing production of various covalent adducts, oxidative damage and DNA single strand break in DNA. To understand roles of histone acetyltransferase GCN5, which protects cells against UV-irradiation, on repair of 4NQO-induced DNA damage, we studied the sensitivity of chicken homozygous DT40 mutants, ΔGCN5, against 4NQO. After 4NQO treatment, the viability of ΔGCN5 was appreciably reduced (to ~25% at 6 hr) as compared to that of wild type DT40. Semiquantitative RT-PCR showed that transcription of DNA polymerase η (*POLH*) gene whose deficiency is responsible for a variant form of xeroderma pigmentosum was drastically down-regulated in ΔGCN5 (to ~25%). However, overexpression of *POLH* could not rescue ΔGCN5 from the enhanced sensitivity to 4NQO, unlike UV-irradiation. Our data suggested that GCN5 participates in control of the sensitivity against 4NQO, and the molecular mechanisms of GCN5-mediated repair of the 4NQO-induced DNA lesions are highly complex.

Key words: DNA repair, DT40, Epigenetics, GCN5, 4-Nitroquinoline 1-oxide

INTRODUCTION

On exposure to DNA-damaging factors such as chemical mutagens, radiation and UV, cells are subjected to DNA damage followed by mutation, cell death and/or carcinogenesis. A typical water-soluble carcinogen 4-nitroquinoline 1-oxide (4NQO) has been used in experimental oral carcinogenesis (Nakahara *et al*., 1957; Kanojia and Vaidya, 2006). Although 4NQO was initially characterized as a UV-mimetic carcinogen, in actuality, it shows more complex effects inducing production of various covalent adducts, oxidative damage and DNA single strand break in DNA (Miao *et al*., 2006). Nucleotide excision repair (NER) system can remove these DNA lesions to protect cells from DNA damage (Balajee and Bohr, 2000; Lans *et al*., 2010). NER system is divided into two different pathways: transcription-coupled repair (TCR) and global genome repair (GGR) (Nouspikel, 2008). DNA damage in the transcribed strand in transcriptional active regions is repaired by TCR, and that in non-transcribed regions of the genome is removed by GGR at relatively slower rate than TCR. 4NQO-induced DNA lesions are mainly repaired by GGR (Snyderwine and Bohr, 1992), while pyrimidine dimers, the most abundant UV-induced DNA lesions, are principally repaired by TCR (Mellon *et al*., 1987). Several cell lines derived from patients of xeroderma pigmentosum (XP) and Cockayne syndrome group B (CSB), autosomal recessive inherited disorders with NER inactivation, showed hypersensitivity to 4NQO (Walker, 1981; Dollery *et al*., 1983; Edwards *et al*., 1987; Moriwaki *et al*., 1993; Prince *et al*., 1999; Muftuoglu *et al*., 2002). In addition, cells from patients of two types of RecQ helicase-deficiency diseases, Werner syndrome and Bloom syndrome, were also remarkably sensitive to 4NQO (Prince *et al*., 1999; Imamura *et al*., 2002; Miao *et al*., 2006). Two RecQ helicase genes, *BLM* (*Rec-*

Correspondence: Hidehiko Kikuchi (E-mail: masakari@shokei-gakuen.ac.jp)

QL2) and *WRN* (*RecQL3*), are responsible for BS and WS, respectively. It is well known that RecQ helicases (RecQL1, BLM, WRN, RecQL4 and RecQL5) participate in DNA double strand break repair and telomere maintenance (Singh *et al*., 2012; Croteau *et al*., 2014). In contrast, interestingly, primary fibroblasts from Rothmund-Thomson syndrome carrying mutations in the *RecQ4* gene showed relative resistance to 4NQO (Jin *et al*., 2008). Thus, various factors are involved in repair of the 4NQO-induced DNA lesions, and the mechanisms of their repair are complicated so far. In particular, the mechanisms of epigenetic controls such as histone acetylation in repair of 4NQO-induced DNA damage remain to be resolved.

Histone acetylation regulated by histone acetyltransferases (HATs) and histone deacetylases (HDACs) epigenetically controls numerous gene expressions (Berger, 2007; Selvi and Kundu, 2009). HATs and HDACs play critical roles in the transcriptional regulation of many genes through histone acetylation and deacetylation, respectively. GCN5, a prototypical HAT, was first identified as a global coactivator and transcription-related HAT (Brownell *et al*., 1996). To investigate physiological roles of GCN5, we generated chicken homozygous DT40 mutants, ΔGCN5, by gene targeting techniques in the chicken B cell line DT40 (Kikuchi *et al*., 2005), which are excellent methods to study physiological roles of various genes (Buerstedde and Takeda, 1991). Studies using ΔGCN5 revealed that GCN5 participates in cell cycle progression at G1/S phase transition (Kikuchi *et al*., 2005), B cell receptor-mediated apoptosis signaling (Kikuchi and Nakayama, 2008), phosphatidylinositol 3-kinase (PI3K)/Akt survival pathway (Kikuchi *et al*., 2011a), superoxide-generating system (Kikuchi *et al*., 2011b), B cell development (Kikuchi *et al*., 2014a), immunoglobulin heavy chain gene expression (Kikuchi *et al*., 2014b), protein kinase Cθ gene expression (Kikuchi *et al*., 2014c) and endoplasmic reticulum stress-induced apoptosis (Kikuchi *et al*., 2015). In particular, interestingly, GCN5 protects cells from UV-irradiation via controlling DNA polymerase η (POLH) gene expression whose deficiency is responsible for XP variant (XPV) (Kikuchi *et al*., 2012).

To investigate the role of GCN5 in regulation of repair of 4NQO-induced DNA lesions, in this study, we studied the sensitivity of ΔGCN5 to 4NQO, and also effects of GCN5-deficiency on various DNA repair-related gene expressions. Our results obtained revealed that GCN5 is involved in the sensitivity of cells in response to 4NQO.

MATERIALS AND METHODS

Materials

4NQO (Sigma-Aldrich, St. Louis, MO, USA), Trizol reagent (Invitrogen, Carlsbad, CA, USA), Ex *Taq* DNA polymerase (Takara Bio, Shiga, Japan), cDNA synthesis kit ReverTra Ace-α (Toyobo, Osaka, Japan) were obtained.

Cell culture and treatments with 4NQO

Generations of ΔGCN5 and human POLH (*hPOLH*)-overexpressing ΔGCN5 were described in our previous reports (Kikuchi *et al*., 2005; Kikuchi *et al*., 2012). DT40 cells and all subclones were cultured essentially as described (Kikuchi *et al.*, 2005). Treatments with DNA-damaging agents were carried out as follows: cells (2×10^6) in 10 mL of culture medium were incubated with 1 μM 4NQO at 37°C. Viable cells were counted by the trypan blue dye exclusion method. DNA fragmentation assay was carried out as described (Kikuchi *et al*., 2012).

Semiquantitative RT-PCR

Total RNA was isolated from DT40 and its subclones using Trizol reagent. Semiquantitative RT-PCR was performed using appropriate sense and antisense primers listed in Supplementary Table 1 as described (Kikuchi *et al*., 2012). Chicken *GAPDH* gene was used as internal control. PCR products were subjected to 1.5% agarose gel electrophoresis. Data obtained by semiquantitative RT-PCR before reaching plateau were analyzed by Multi Gauge software (densitometric analysis mode) using a luminescent image analyzer LAS-3000 (Fujifilm, Tokyo, Japan).

Statistical analysis

Results of PCR are presented as averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated with Student's *t* test.

RESULTS AND DISCUSSION

GCN5-deficiency enhances the sensitivity to 4NQO

First, to know influences of GCN5-deficiency on 4NQO-induced cytotoxicity, we examined effects of 4NQO treatment on cell viability and DNA fragmentation in ΔGCN5 (clone 1~3) and wild type DT40. After 4NQO treatment, the viability of three independent ΔGCN5 clones was appreciably reduced (to ~25% at 6 hr) as compared to that of wild type DT40 (to ~70% at 6 hr)

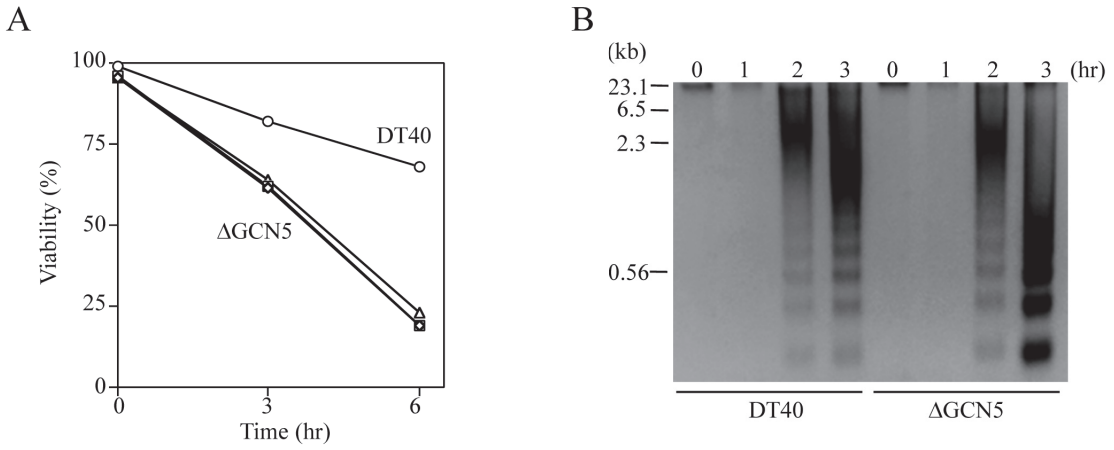

Fig. 1. Influences of GCN5-deficiency on sensitivity to 4NQO. (A) Cell viability. After 4NQO-treatment (1 μM), wild type DT40 (circles) and three independent ΔGCN5 clones 1, 2 and 3 (squares, triangles and lozenges) were cultured at 37°C up to 6 hr. Viable cells were counted by the trypan blue dye exclusion method. Data represent the average of three separate experiments. (B) DNA fragmentation. After 1 μM 4NQO-treatment, the cells (wild type DT40 and ΔGCN5 clone 1) were cultured at 37°C for 0, 1, 2 and 3 hr. DNAs were isolated from the cells and analyzed by 1.5% agarose gel electrophoresis. The sizes of λ-DNA digested with *Hin*dIII are indicated in kilobase pairs.

(Fig. 1A). In addition, the DNA fragmentation in ΔGCN5 clone 1 was more prominent compared to that of wild type DT40 (Fig. 1B). Similar results were obtained in two other ΔGCN5 clones (data not shown). In contrast, as shown in our previous report (Kikuchi *et al*., 2012), GCN5-deficiency showed insignificant influences on the sensitivity to various DNA-damaging agents (aphidicolin, bleomycin, cisplatin, camptothecin, etoposide, mitomycin C and methyl methanesulfonate). These results suggested that GCN5 is necessary for cell survival against 4NQO treatment.

GCN5-deficiency affects transcription of various DNA repair-related genes

To study the influences of GCN5-deficiency on expressions of DNA repair-related genes; Ataxia telangiectasia mutated (*ATM*)*, BLM,* breast cancer susceptibility gene 1 and 2 (*BRCA1* and *2*), DNA-damage binding protein 1 (*DDB1*), excision repair cross-complementing 6 and 8 (*ERCC6* and *8*), Fanconi anemia complementation group D2 (*FANCD2*), Nijmegen breakage syndrome 1 (*NBS1*), proliferation cell nuclear antigen (*PCNA*), DNA polymerase δ (*POLD*), DNA polymerase ε (*POLE*), *POLH*, DNA polymerase κ (*POLK*), DNA polymerase ζ (*POLZ*), radiation-sensitive 18 (*RAD18*), replication factor 1 (*RFC1*), replication protein A 1~3 (*RPA1~3*), *WNR*, *XPA~XPG*, we carried out semiquantitative RT-PCR on total RNAs prepared from three independent ΔGCN5 clones and wild type DT40 (Fig. 2). As expected, GCN5-deficiency showed significant influence on transcription of various DNA repair-related genes; *PCNA* (to ~55%), *POLE* (to ~65%), *RecQL1* (to ~50%), *RFC1* (to ~75%), *RPA1* (to ~65%), *RPA3* (to ~75%), *WNR* (to ~140%) and *XPF* (to ~75%). In particular, transcription of *POLH* gene whose deficiency is responsible for XPV was significantly down-regulated in ΔGCN5 (to ~25%). These findings suggested that the remarkable decrease in gene expression of *POLH* in ΔGCN5 may result in accelerated sensitivity to 4NQO as well as UV-irradiation (Kikuchi *et al.*, 2012).

Overexpression of hPOLH cannot rescue ΔGCN5 from the enhanced sensitivity to 4NQO

As reported in our previous paper, overexpression of *hPOLH* in ΔGCN5 dramatically reversed the sensitivity to UV-irradiation of ΔGCN5 to almost the same level of wild type DT40 (Kikuchi *et al*., 2012). To investigate whether or not the down-regulation of *POLH* caused the enhanced sensitivity to 4NQO as well as UV-irradiation in ΔGCN5, ΔGCN5/*hPOLH* cells, *hPOLH*-overexpressing ΔGCN5 cells (Kikuchi *et al.*, 2012), were treated with 4NQO. However, overexpression of *hPOLH* could not rescue ΔGCN5 from the enhanced sensitivity to 4NQO at all (Fig. 3). These data suggested that remarkable decrease of *POLH* did not contribute to the high sensitivity to 4NQO in ΔGCN5.

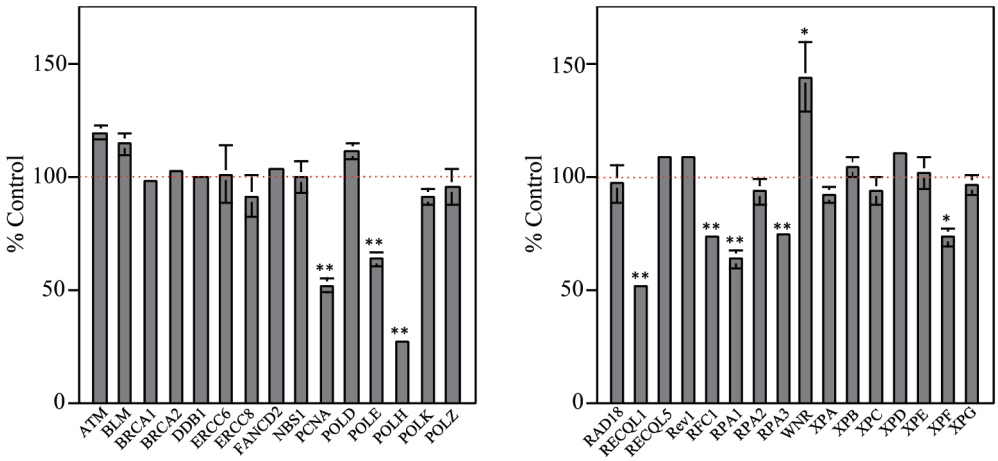

Fig. 2. Influences of GCN5-deficiency on expressions of various DNA repair-related genes. Total RNAs were extracted from wild type DT40 and three independent ΔGCN5 clones, and mRNA levels of DNA repair-related genes found in chicken were determined by semiquantitative RT-PCR using appropriate primers (see Supplementary Table 1). Chicken *GAPDH* gene was used as internal control. Data represent averages of three independent ΔGCN5 clones (gray bars), and indicated as percentages of control values (100%) obtained from wild type DT40 cells. Error bars indicate standard deviation. Statistical differences were calculated using Student's *t* test. *, $p < 0.05$; **, $p < 0.01$.

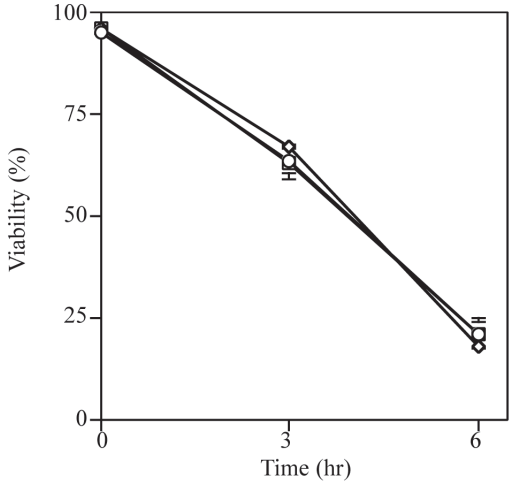

Fig. 3. Influences of overexpression of *hPOLH* in ΔGCN5 on sensitivity to 4NQO-treatment. After 4NQO-treatment (1 μM), wild type DT40 (circles) and two independent ΔGCN5/*hPOLH* clones-1 and -2 (squares and lozenges) were cultured at 37°C up to 6 hr. Viable cells were counted by the trypan blue dye exclusion method. Data represent averages of three separate experiments. Error bars indicate standard deviation.

Concluding remarks

Our findings in this study reveal that GCN5 participates in resistance against 4NQO in vertebrate cells. The GCN5-deficient DT40 mutants, ΔGCN5, established by us showed a remarkably enhanced sensitivity to 4NQO but not to other DNA-damaging agents (Fig. 1), suggesting that GCN5-deficiency may influence the expressions of DNA repair-related genes, which are involved in repair of the 4NQO-induced DNA lesions. Therefore, we carried out semiquantitative RT-PCR to study influences of GCN5-deficiency on expressions of various DNA repair-related genes. As expected, GCN5-deficiency caused influences to some extent on transcriptions of various genes tested (Fig. 2). It is noteworthy that transcription of *POLH* gene whose decrease caused remarkable attenuation of UV-tolerance in ΔGCN5 (Kikuchi *et al*., 2012) was drastically down-regulated (to ~25%). However, unexpectedly, overexpression of *hPOLH* could not rescue ΔGCN5 from the enhanced sensitivity to 4NQO (Fig. 3), unlike UV. Since deficiency of *Rev1*, which is involved in translesion synthesis (TLS) the same as *POLH*, showed no effect on the sensitivity to 4NQO (Sakai *et al.*, 2003), TLS-related genes may not contribute to repair of the 4NQO-induced DNA lesions. Changes on transcriptions of other DNA repair-related genes (*PCNA, POLE, RecQL1, RFC1, RPA1, RPA3, WNR* and *XPF*) were relatively moderate, and were insufficient for understanding the mechanisms of the drastic enhanced sensitivity of ΔGCN5 against 4NQO. On the other hand, no changes were detected in the mRNA levels of *XPA~E*, *XPG* and *ERCC6* whose deficiency is responsible for CSB. As mentioned above, cell lines derived from patients of XP

and CSB showed hypersensitivities to 4NQO (Walker, 1981; Dollery *et al.*, 1983; Edwards *et al.*, 1987; Moriwaki *et al*., 1993; Prince *et al*., 1999; Muftuoglu *et al*., 2002). However, our data suggested that the hypersensitivity of ΔGCN5 against 4NQO is not caused by down-regulation of these genes. Transcripts of *RecQL4* gene, unfortunately, could not detected by RT-PCR using several primers. In addition, there is the possibility that the unidentified factors may participate in repair of the 4NQO-induced DNA lesions. Identification of the target genes of GCN5 involved in repair of the 4NQO-induced DNA lesions should be done in the future.

Another possibility is that GCN5-deficiency-induced hypoacetylation surrounding the 4NQO-induced DNA lesions enhances the sensitivity to 4NQO. As is well known, GCN5 promotes histone acetylation resulting in recruitment of DNA repair-related protein factors to damaged sites and efficient DNA repair (Brand *et al*., 2001; Martinez *et al*., 2001; Teng *et al*., 2002; Yu *et al*., 2005; Gamper and Roeder, 2008; Shimada *et al*., 2008; Guo *et al*., 2011). In fact, GCN5-deficiency in DT40 cells led to remarkable decreased bulk acetylation level of Lys-9 residue of histone H3 (Kikuchi *et al.*, 2005). Such changes in the pattern of histone acetylation can influence the sensitivity to 4NQO. Perhaps, changes in the expression of some DNA repair-related genes and histone acetylation levels might cooperatively bring about the enhanced sensitivity against 4NQO. Unfortunately, we have no data proving this hypothesis. In addition, the reason why ΔGCN5 showed an enhanced sensitivity to 4NQO but not to other DNA-damaging agents still remains unexplained. Further studies will be needed to clarify molecular mechanisms of GCN5-mediated repair of the 4NQO-induced DNA lesions.

Nonetheless, it is quite certain that GCN5 participates in control of the sensitivity against 4NQO. As mentioned in our previous study, GCN5 is involved in regulation of various cell death progressions. For example, GCN5-deficiency causes acceleration of apoptosis induced by hydrogen peroxide (Kikuchi *et al*., 2011b) and UV-irradiation (Kikuchi *et al*., 2012), while it suppresses apoptotic cell death mediated by B cell receptor-signaling (Kikuchi and Nakayama, 2008) and endoplasmic reticulum stress (Kikuchi *et al*., 2015). Moreover, GCN5-deficiency in mice led to early embryonic lethality with increased apoptosis in mesodermal lineages (Xu *et al*., 2000; Yamauchi *et al*., 2000). Thus, GCN5 is one of the most significant epigenetic supervisor in the decision between cell life and cell death. Studies on GCN5 functions will become more important for epigenetic control of life and death in vertebrate cells.

ACKNOWLEDGMENTS

We thank H. Madhyastha and R. Madhyastha for editorial reading of the manuscript. This work was supported in part by Grant-in-Aid for Scientific Research from the Ministry of Education, Culture, Sports, Science and Technology of Japan (No. 25430170, to H. K. and No. 15K09671, to F. K.). This work was also partly supported by the Grant for Joint Research Project of the Institute of Medical Science, the University of Tokyo (2013-230, to H. K.).

Conflict of interest---- The authors declare that there is no conflict of interest.

REFERENCES

Balajee, A.S. and Bohr, V.A. (2000): Genomic heterogeneity of nucleotide excision repair. Gene, **250**, 15-30.

Berger, S.L. (2007): The complex language of chromatin regulation during transcription. Nature, **447**, 407-412.

Brand, M., Moggs, J.G., Oulad-Abdelghani, M., Lejeune, F., Dilworth, F.J., Stevenin, J., Almouzni, G. and Tora, L. (2001): UV-damaged DNA-binding protein in the TFTC complex links DNA damage recognition to nucleosome acetylation. EMBO J., **20**, 3187-3196.

Brownell, J.E., Zhou, J., Ranalli, T., Kobayashi, R., Edomondson, D.G., Roth, S.Y. and Allis, C.D. (1996): Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell, **84**, 843-851.

Buerstedde, J.M. and Takeda, S. (1991): Increased ration of targeted to random integration after transfection of chicken B cell lines. Cell, **67**, 179-188.

Croteau, D.L., Popuri, V., Opresko, P.L. and Bohr, V.A. (2014): Human RecQ helicases in DNA repair, recombination, and replication. Annu. Rev. Biochem., **83**, 519-552.

Dollery, A.A., Melvin, W.T., Keir, H.M. and Harris, W.J. (1983): Repair of 4-nitroquinoline-1-oxide induced DNA damage in normal human cells and cells from classical and variant xeroderma pigmentosum. Mutat. Res., **112**, 33-46.

Edwards, S., Fielding, S. and Waters, R. (1987): The response to DNA damage induced by 4-nitroquinoline-1-oxide or its 3-methyl derivative in xeroderma pigmentosum fibroblasts belonging to different complementation groups: evidence for different epistasis groups involved in the repair of large adducts in human DNA. Carcinogenesis, **8**, 1071-1075.

Gamper, A.M. and Roeder, R.G. (2008): Multivalent binding of p53 to the STAGA complex mediates coactivator recruitment after UV damage. Mol. Cell Biol., **28**, 2517-2527.

Guo, R., Chen, J., Mitchell, D.L. and Johnson, D.G. (2011): GCN5 and E2F1 stimulate nucleotide excision repair by promoting H3K9 acetylation at sites of damage. Nucleic. Acids Res., **39**, 1390-1397.

Imamura, O., Fujita, K., Itoh, C., Takeda, S., Furuichi, Y. and Matsumoto, T. (2002): Werner and Bloom helicases are involved in DNA repair in a complementary fashion. Oncogene, **21**, 954-963.

Jin, W., Liu, H., Zhang, Y., Otta, S.K., Plon, S.E. and Wang, L.L. (2008): Sensitivity of RECQL4-deficient fibroblasts from Rothmund-Thomson syndrome patients to genotoxic agents. Hum. Genet., **123**, 643-653.

Kanojia, D. and Vaidya, M.M. (2006): 4-Nitroquinoline-1-oxide induced experimental oral carcinogenesis. Oral Oncol., **42**, 655-667.

Kikuchi, H., Takami, Y. and Nakayama, T. (2005): GCN5: a supervisor in all-inclusive control of vertebrate cell cycle progression through transcription regulation of various cell cycle-related genes. Gene, **347**, 83-97.

Kikuchi, H. and Nakayama, T. (2008): GCN5 and BCR signalling collaborate to induce pre-mature B cell apoptosis through depletion of ICAD and IAP2 and activation of caspase activities. Gene, **419**, 48-55.

Kikuchi, H., Kuribayashi, F., Takami, Y., Imajoh-Ohmi, S. and Nakayama, T. (2011a): GCN5 regulates the activation of PI3K/Akt survival pathway in B cells exposed to oxidative stress via controlling gene expressions of Syk and Btk. Biochem. Biophys. Res. Commun., **405**, 657-661.

Kikuchi, H., Kuribayashi, F., Kiwaki, N., Takami, Y. and Nakayama, T. (2011b): GCN5 regulates the superoxide-generating system in leukocytes via controlling gp91-phox gene expression. J. Immunol., **186**, 3015-3022.

Kikuchi, H., Kuribayashi, F., Imajoh-Ohmi, S., Nishitoh, H., Takami, Y. and Nakayama, T. (2012): GCN5 protects vertebrate cells against UV-irradiation via controlling gene expression of DNA polymerase η. J. Biol. Chem., **287**, 39842-39849.

Kikuchi, H., Nakayama, M., Kuribayashi, F., Imajoh-Ohmi, S., Nishitoh, H., Takami, Y. and Nakayama, T. (2014a): GCN5 is essential for IRF-4 gene expression followed by transcriptional activation of Blimp-1 in immature B cells. J. Leukoc. Biol., **95**, 399-404.

Kikuchi, H., Nakayama, M., Kuribayashi, F., Imajoh-Ohmi, S., Nishitoh, H., Takami, Y. and Nakayama, T. (2014b): GCN5 is involved in regulation of immunoglobulin heavy chain gene expression in immature B cells. Gene, **544**, 19-24.

Kikuchi, H., Nakayama, M., Kuribayashi, F., Imajoh-Ohmi, S., Nishitoh, H., Takami, Y. and Nakayama, T. (2014c): Protein kinase Cθ gene expression is oppositely regulated by GCN5 and EBF1 in immature B cells. FEBS Lett., **588**, 1739-1742.

Kikuchi, H., Kuribayashi, F., Mimuro, H., Imajoh-Ohmi, S., Nakayama, M., Takami, Y., Nishitoh, H. and Nakayama, T. (2015): Lack of GCN5 remarkably enhances the resistance against prolonged endoplasmic reticulum stress-induced apoptosis through up-regulation of Bcl-2 gene expression. Biochem. Biophys. Res. Commun., **463**, 870-875.

Lans, H., Marteijn J.A., Schumacher, B., Hoeijmakers, J.H., Jansen, G. and Vermeulen, W. (2010): Involvement of grobal genome repair, transcription coupled repair, and chromatin remodeling in UV DNA damage response changes during development. PLoS Genet., **6**, e1000941.

Mellon, I., Spivak, G. and Hanawalt, P.C. (1987): Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. Cell, **51**, 241-249.

Miao, Z.H., Rao, V.A., Agama, S., Antony, S., Kohn, K.W. and Pommier, Y. (2006): 4-Nitroquinoline-1-oxide induces the formation of cellular topoisomerase I-DNA cleavage complexes. Cancer Res., **66**, 6540-6545.

Moriwaki, S., Nishigori, C., Teramoto, T., Tanaka, T., Kore-eda, S., Takebe, H. and Imamura, S. (1993): Absence of DNA repair deficiency in the confirmed heterozygotes of xeroderma pigmentosum group A. J. Invest. Dermatol., **101**, 69-72.

Martinez, E., Palhan, V.B., Tjernberg, A., Lymar, E.S., Gamper, A. M., Kundu, T.K., Chait, B.T. and Roeder, R.G. (2001): Human STAGA complex is a chromatin-acetylating transcription coactivator that interacts with pre-mRNA splicing and DNA damage-binding factors *in vivo*. Mol. Cell Biol., **21**, 6782-6795.

Muftuoglu, M., Selzer, R., Tuo, J., Brosh, Jr.R.M. and Bohr, V.A. (2002): Phenotypic consequences of mutations in the conserved motifs of the putative helicase domain of the human Cockayne Syndrome Groupe B gene. Gene, **283**, 27-40.

Nakahara, W., Fukuoka, F. and Sugimura, T. (1957): Carcinogenic action of 4-nitroquinoline-N-oxide. Gan, **48**, 129-137.

Nouspikel, T. (2008): Nucleotide excision repair and neurological diseases. DNA Repair, **7**, 1155-1167.

Prince, P.R., Ogburn, C.E., Moser, M.J., Emond, M.J., Martin, G.M. and Monnat Jr, R.J. (1999): Cell fusion corrects the 4-nitroquinoline 1-oxide sensitivity of Werner syndrome fibroblast cell lines. Hum. Genet., **105**, 132-138.

Sakai, W., Wada, Y., Naoi, Y., Ishii, C. and Inoue, H. (2003): Isolation and genetic characterization of the *Neurospora crassa REV1* and *REV7* homologs: evidence for involvement in damage-induced mutagenesis. DNA Repair, **2**, 337-346.

Selvi, R.B. and Kundu, T.K. (2009): Reversible acetylation of chromatin: implication in regulation of gene expression, disease and therapeutics. Biotechnol. J., **4**, 375-390.

Shimada, M., Niida, H., Zineldeen, D. H., Tagami, H., Tanaka, M., Saito, H. and Nakanishi, M. (2008): Chk1 is a histone H3 threonine 11 kinase that regulates DNA damage-induced transcriptional repression. Cell, **132**, 221-232.

Singh, D.K., Ghosh, A.K., Croteau, D.L. and Bohr, V.A. (2012): RecQ helicases in DNA double strand break repair and telomere maintenance. Mutat. Res., **736**, 15-24.

Snyderwine, E.G. and Bohr, V.A. (1992): Gene- and strand-specific damage and repair in Chinese hamster ovary cells treated with 4-nitroquinoline 1-oxide. Cancer Res., **52**, 4183-4189.

Teng, Y., Yu, Y. and Waters, R. (2002): The Saccharomyces cerevisiae histone acetyltransferase Gcn5 has a role in the photoreactivation and nucleotide excision repair of UV-induced cyclobutane pyrimidine dimers in the MFA2 gene. J. Mol. Biol., **316**, 489-499.

Walker, I.G. (1981): Alkaline sucrose sedimentation analysis as an indicator of repair capability of xeroderma pigmentosum fibroblasts for 4-nitroquinoline-1-oxide damage. Carcinogenesis, **2**, 691-695.

Xu, W., Edmondson, D.G., Evrard, Y.A., Wakamiya, M., Behringer, R.R. and Roth, S.Y. (2000): Loss of Gcn5l2 leads to increased apoptosis and mesodermal defects during mouse development. Nat. Genet., **26**, 229-232.

Yamauchi, T., Yamauchi, J., Kuwata, T., Tamura, T., Yamashita, T., Bae, N., Westphal, H., Ozato, K. and Nakatani, Y. (2000): Distinct but overlapping roles of histone acetylase PCAF and of the closely related PCAF-B/GCN5 in mouse embryogenesis. Proc. Natl. Acad. Sci. USA, **97**, 11303-11306.

Yu, Y., Teng, Y., Liu, H., Reed, S.H. and Waters, R. (2005): UV irradiation stimulates histone acetylation and chromatin remodeling at a repressed yeast locus. Proc. Natl. Acad. Sci. USA, **102**, 8650-8655.