Current Topics in Biochemical Research
Vol. 19, 2018

Review

Gene targeting techniques reveal that histone acetyltransferase GCN5 has various inherent physiological functions in chicken DT40 cells

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ABSTRACT

The DNA-histone complex in eukaryotic nuclei is called chromatin. In chromatin, histones (H1, H2A, H2B, H3 and H4), which are low-molecular-weight proteins, tightly bind with DNA, because all the histones are positively charged and DNA is negatively charged. The electrostatic DNA-histone interactions are significantly involved in the formation of the chromatin structure. Alterations in the chromatin structure are essential for access of various transcription factors to chromosomal DNA in eukaryotic cells. Histone acetyltransferases (HATs) catalyze acetylation of core histones resulting in attenuation of the electrostatic DNA-histone interactions. In contrast, histone deacetylases (HDACs) remove acetyl groups from the acetylated histones resulting in recovery of the interactions. Control of histone acetylation/deacetylation levels by HATs and HDACs is a significant mechanism for transcriptional regulation through alterations in the chromatin structure. General control non-depressible 5 (GCN5) belonging to GCN5-family HATs, which consist of GCN5 and p300/CBP-associated factor (PCAF), was first identified as a global coactivator and transcription-related HAT. GCN5 mainly catalyzes acetylation of histones H2B, H3 and H4 that is an important modification for epigenetic transcriptional activation. The importance of GCN5 is also emphasized by some studies revealing that GCN5-knockout mice die during embryogenesis. In this review article concerning our own past studies, we describe various inherent physiological functions of GCN5, which were revealed by us using gene targeting techniques in chicken immature B cell line DT40 cells: (i) regulation of cell cycle and cell proliferation, (ii) regulation of B cell receptor-mediated apoptosis related to negative selection of B cells, (iii) regulation of superoxide generation in leukocytes, (iv) regulation of response to hydrogen peroxide-induced oxidative stress, (v) regulation of UV-tolerance, (vi) regulation of B cell differentiation, and (vii) regulation of endoplasmic reticulum stress-induced apoptosis. Thus, we show that gene targeting techniques in DT40 cells can be a powerful tool for the functional analysis of GCN5. Our results, together with enormous previous data, significantly contribute to the elucidation of inherent physiological functions of GCN5.

KEYWORDS: DT40, GCN5, gene targeting techniques, histone acetyltransferase.

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INTRODUCTION

Chromatin is the DNA-protein complex in eukaryotic nuclei. The major proteins in chromatin are called histones. Histones, which are positively charged and low-molecular-weight nuclear proteins, are classified into linker histone H1, and core histones H2A, H2B, H3 and H4. Core histones H2A, H2B, H3 and H4 are known as components of the histone octamer. Because DNA is negatively charged due to its phosphate groups, the histone octamer tightly binds with DNA to form nucleosome, a fundamental unit of chromatin. Histone acetyltransferases (HATs) catalyze acetylation of core histones resulting in attenuation of the electrostatic DNA-histone interactions, and histone deacetylases (HDACs) remove acetyl groups from the acetylated histones resulting in recovery of the interactions [1-3]. Because acetylation and deacetylation of core histones regulate various DNA-related cell functions, such as transcription, DNA replication, DNA repair and so on, through alterations in the chromatin structure in eukaryotic cells, those are well known as one of the most common and important epigenetic mechanisms [1-4]. Therefore, to clarify the acetylation and deacetylation in detail, it is important to elucidate the physiological functions of HATs and HDACs. However, because there are various members of HATs and HDACs in vertebrate cells, the respective functions of them remain poorly defined due to their vast and complicated compensation.

General control non-depressible 5 (GCN5) was first identified as a global coactivator and transcription-related HAT in Tetrahymena [5]. GCN5 is a member of the A-type HATs as well as GCN5-related N-acetyltransferase superfamily, and its catalytic domain is well conserved from yeast to mammalians. GCN5 catalyzes acetylation of core histones H2B, H3 and H4, resulting in not only attenuation of DNA-histone interaction but also formation of epigenetic tags for transcriptional activation [4, 6, 7]. GCN5 deficiency in mice led to early embryonic lethality with increased apoptosis in mesodermal lineages [8]. In addition, loss of GCN5 in mouse embryonic stem cells invoked a cell-autonomous pathway of cell death [9]. These results suggest that GCN5 is essential for normal development of vertebrates. However, the inherent functions of GCN5 remain to be elucidated in vertebrates. Therefore, to further investigate inherent physiological roles of GCN5, we generated chicken homozygous DT40 mutants, *GCN5*$^{-/-}$, devoid of two GCN5 alleles using gene targeting techniques [10, 11]. In this review article, we describe various inherent physiological functions of GCN5 using *GCN5*$^{-/-}$ mutant cells.

GCN5 regulates cell cycle and cell proliferation

We first examined the influence of GCN5 deficiency on the cell growth [11]. While the doubling times of wild-type DT40 cells were about 12 h [12], those of *GCN5*$^{-/-}$ cells were about 17 h. To determine why *GCN5*$^{-/-}$ cells showed the delayed growth rate, we measured the cellular DNA content by flow cytometry after pulse 5-bromodeoxyuridine (BrdUrd)-labeling. Certain proportions of wild-type DT40 cells were detected with a high BrdUrd uptake in S phase (~55%) as compared with G2/M phase (~22%) and G1 phase (~21%). In contrast, the proportions of *GCN5*$^{-/-}$ cells were decreased (to ~42%) in S phase and increased (to ~30%) in G1 phase. Moreover, FACS analyses of synchronized wild-type DT40 and *GCN5*$^{-/-}$ cells revealed that GCN5-deficiency suppresses the cell cycle progression through inhibition of the G1 to S phase transition. Next, to determine whether or not GCN5-deficiency affects the transcriptions of various cell cycle-related genes, we performed semiquantitative reverse transcription-polymerase chain reaction (RT-PCR) on total RNAs prepared from wild-type DT40 and *GCN5*$^{-/-}$ cells. The steady-state mRNA levels of various cell cycle-promoting factors (cdc25B, cyclin A, cyclin D3, DP-2, E2F-1, E2F-3, E2F-4, E2F-6 and PCNA) in *GCN5*$^{-/-}$ cells were moderately or slightly decreased as compared with those in wild-type DT40 cells. In addition, GCN5-deficiency led to decreased bulk acetylation levels of Lys-16 of histone H2B (H2BK16) and Lys-9 of histone H3 (H3K9). These results suggest that GCN5 behaves as a supervisor, which comprehensively regulates normal cell cycle progression through controlling transcriptions of genes encoding various cell cycle-promoting factors through alterations in the chromatin structure (Fig. 1).

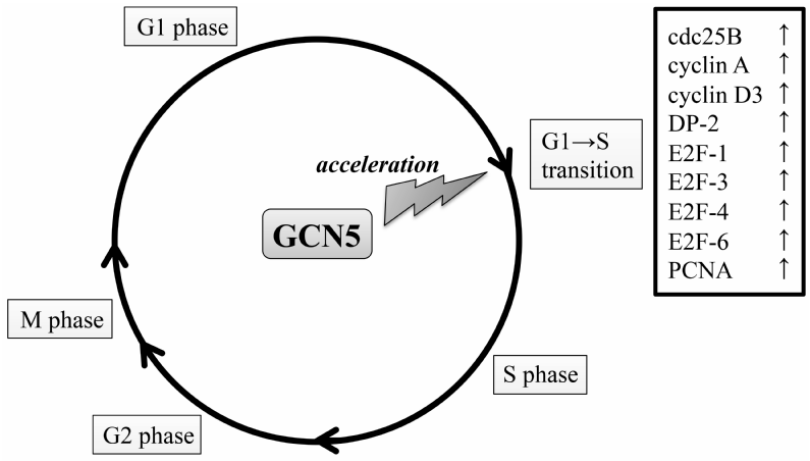

Fig. 1. GCN5 participates in the regulation of cell cycle progression. The mRNA levels of various cell cycle-promoting factors (cdc25B, cyclin A, cyclin D3, DP-2, E2F-1, E2F-3, E2F-4, E2F-6 and PCNA) were moderately or slightly decreased in *GCN5*$^{-/-}$ cells as compared with those in DT40 cells. The doubling times of DT40 and *GCN5*$^{-/-}$ cells were 12 and 17 hrs, respectively. These results suggest that GCN5 comprehensively regulates normal cell cycle progression through up-regulation of gene expressions of various cell cycle-promoting factors.

GCN5 regulates B cell receptor-mediated apoptosis related to negative selection of B cells

The antigen binding to the B cell receptor (BCR) of immature B cells induces apoptosis, while the antigen binding to the BCR of mature B cells promotes their activation and proliferation [13-15]. The former reaction known as negative selection is the mechanism to eliminate B cells that react with self-antigen, and therefore it contributes to escape from auto-immune diseases [16].

To investigate effects of GCN5-deficiency on BCR-mediated apoptosis, both wild-type DT40 and *GCN5*$^{-/-}$ cells were treated with phorbol 12-myristate 13-acetate (PMA)/ionomycin, which mimics the BCR stimulation [17]. As expected, the PMA/ionomycin treatment induced apoptosis in wild-type DT40 cells, and their viability was dramatically reduced. In contrast, very surprisingly, the PMA/ionomycin-induced apoptosis was not caused in *GCN5*$^{-/-}$ cells, and their viability was not changed during cultivation up to 36 h. Further analyses revealed that concerning BCR-mediated stimulation, GCN5 promotes apoptosis in immature B cells through both depletion of ICAD and IAP2 (inhibitors for apoptosis) activities and elevation of caspase-3 (effector caspase) and caspase-8 (initiator caspase) activities, resulting in increased activity of CAD (effector for apoptosis). These results suggest that GCN5 acts as a key factor in negative selection of B cells and also in auto-immune diseases (Fig. 2).

GCN5 regulates superoxide generation in leukocytes

The NOX family, which acts as an active site of the reactive oxygen-generating NADPH oxidases, consists of 7 proteins: NOX1-5 proteins and DUOX1 and 2 proteins [18]. Among them, NOX2 (gp91-phox) protein is a member of the superoxide anion (O_2^-)-generating system in leukocytes (phagocytes and B cells) [19, 20]. This system is an important mechanism of innate immune response against various infections in phagocytes (macrophages, neutrophils and eosinophils) and is involved in signal transduction mediated by various biological signals in various different cells including B cells. Five specific proteins, NOX2 (gp91-phox), p22-phox, p40-phox, p47-phox and p67-phox proteins, are essential for this system. NOX2 (gp91-phox) and p22-phox proteins are large and small subunits of cytochrome b558 in plasma membrane, respectively. On the other hand, p40-phox, p47-phox and p67-phox proteins are cytosolic proteins [18]. Activation of the O_2^--generating system requires stimulus-induced membrane translocation of these cytosolic proteins and small G protein

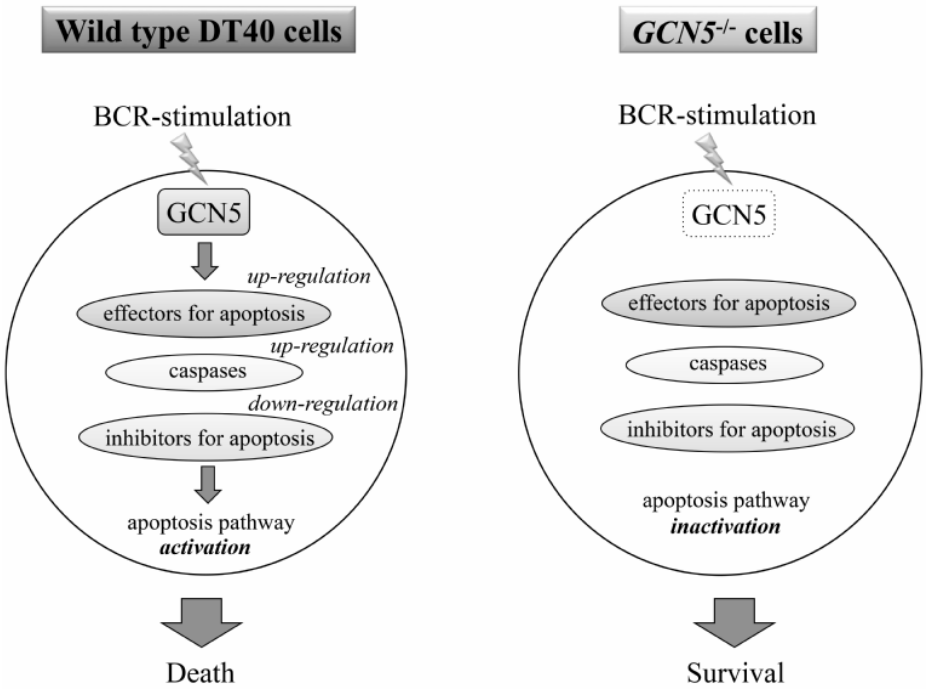

Fig. 2. GCN5 participates in the regulation of B cell receptor-mediated apoptosis. The PMA/ionomycin treatment, which mimics the BCR stimulation, induced apoptosis in DT40 cells but not in *GCN5*$^{-/-}$ cells. The viability of the former was dramatically reduced but that of the latter was not changed. Concerning BCR-mediated stimulation, GCN5 promotes apoptosis in immature B cells through depletion of ICAD and IAP2 (inhibitors for apoptosis) activities and elevation of caspase-3 (effector caspase) and caspase-8 (initiator caspase) activities, resulting in increased activity of effectors for apoptosis. These results suggest that GCN5 acts as a key factor in the negative selection of B cells.

Rac [18]. The importance of this system is emphasized by a genetic disorder known as chronic granulomatous disease (CGD) [21]. Because phagocytes and B cells in CGD patients cannot produce O_2^- upon bacterial and fungal infections, they are subjected to frequent severe bacterial and fungal infections.

To investigate effects of GCN5-deficiency on O_2^--generating activity, both wild-type DT40 and *GCN5*$^{-/-}$ cells were stimulated with PMA [22]. As expected, wild-type DT40 cells generated O_2^- when stimulated with PMA. In contrast, very surprisingly, *GCN5*$^{-/-}$ cells only produced a negligible level of O_2^-, indicating that GCN5 deficiency causes complete prevention of the O_2^--generating system. Next, to know the influence of GCN5 deficiency on transcriptions of four genes responsible for CGD (NOX2 (gp91-phox), p22-phox, p47-phox and p67-phox), semiquantitative RT-PCR was carried out on total RNAs prepared from wild-type DT40 and *GCN5*$^{-/-}$ cells. Interestingly, transcription of the NOX2 (gp91-phox) gene was drastically down-regulated in *GCN5*$^{-/-}$ cells. These results indicated that GCN5 up-regulates transcription of the NOX2 (gp91-phox) gene. Further, chromatin immunoprecipitation (ChIP) assay using *in vitro* differentiation system of human monoblast U937 cells revealed not only that interaction of GCN5 with the NOX2 (gp91-phox) gene promoter is significantly accelerated, but also that GCN5 preferentially elevates acetylation levels of H2BK16 and H3K9 surrounding the promoter. These results suggest that GCN5 as a supervisor regulates the O_2^--generating system in leukocytes through controlling the NOX2 (gp91-phox) gene expression (Fig. 3). As is well known, about 70% of CGD results from the defective NOX2 (gp91-phox) gene including its promoter. Therefore, our findings may be useful in the development of treatment for CGD.

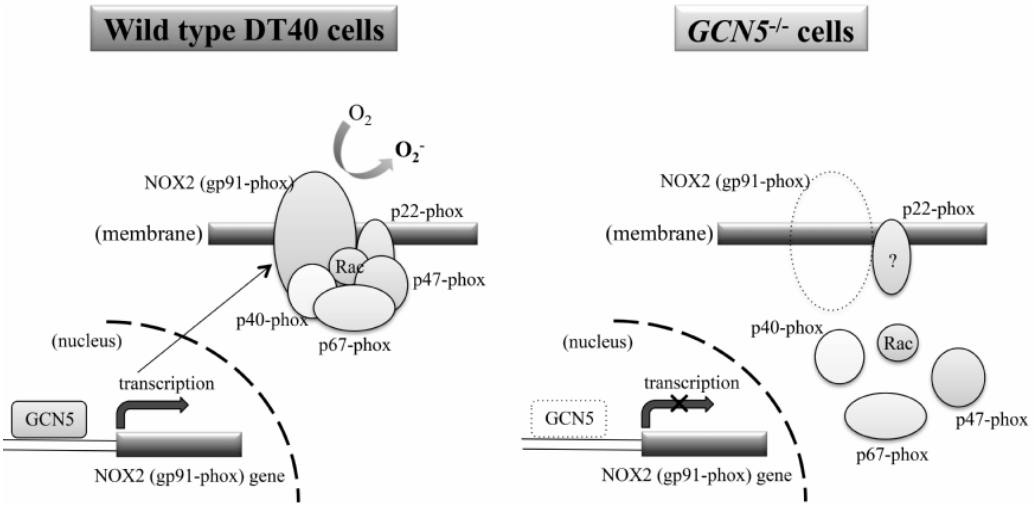

Fig. 3. GCN5 participates in the regulation of O_2^- generation in leukocytes through controlling gp91-phox gene expression. The PMA treatment showed that DT40 cells generate O_2^- but *GCN5*$^{-/-}$ cells produce an undetectable level of O_2^-, indicating that GCN5 deficiency causes complete prevention of the O_2^--generating system. To know the influence of GCN5 deficiency on transcriptions of NOX2 (gp91-phox), p22-phox, p47-phox and p67-phox genes, semiquantitative RT-PCR was carried out on total RNAs prepared from DT40 and *GCN5*$^{-/-}$ cells. Transcription of the NOX2 (gp91-phox) gene was drastically down-regulated in *GCN5*$^{-/-}$ cells, indicating that GCN5 up-regulates its transcription. ChIP assay using human U937 cells revealed that interaction of GCN5 with gp91-phox gene promoter is significantly accelerated, and GCN5 preferentially elevates acetylation levels of H2BK16 and H3K9 surrounding the promoter. These results suggest that GCN5 regulates the O_2^--generating system through controlling gp91-phox gene expression in leukocytes.

GCN5 regulates response to hydrogen peroxide-induced oxidative stress

Mediators of oxidative stress such as reactive oxygen species (ROS) triggers the activation of various intracellular signaling including cell survival and death pathways. For example, ROS including hydrogen peroxide (H_2O_2) activates phosphatidylinositol 3-kinase (PI3K)/Akt8 survival pathway [23, 24]. On stimulation with exogenous H_2O_2, two protein-tyrosine kinases, spleen tyrosine kinase (Syk) and Bruton's tyrosine kinase (BTK) activate the PI3K/Akt8 survival pathway in B cells [25, 26]. Semiquantitative RT-PCR showed that gene expressions of Syk and Btk in *GCN5*$^{-/-}$ cells are remarkably decreased as compared with those in wild-type DT40 cells [27]. Based on these findings, wild-type DT40 and *GCN5*$^{-/-}$ cells were treated with H_2O_2. As expected, in the presence of H_2O_2, the viability of *GCN5*$^{-/-}$ cells (~55% at 24 h) was markedly decreased as compared with that of wild-type DT40 cells (~85% at 24 h). Moreover, ChIP assay revealed that GCN5 binds to proximal 5'-upstream regions of the Syk and Btk genes. These results suggest that GCN5 regulates the activation of PI3K/Akt8 survival pathway in B cells exposed to endogenous H_2O_2 through controlling gene expressions of Syk and Btk (Fig. 4).

GCN5 regulates UV-tolerance

On UV-irradiation, cells are subjected to DNA damage followed by cell death, mutation and carcinogenesis [28]. UV produces specific DNA damages, cyclobutane pyrimidine dimers (CPDs) and pyrimidine(6-4)pyrimidine photo-products (6-4PDs), at dipyrimidine sites in DNA [28]. Vertebrate cells have DNA repair system such as nucleotide excision repair and translesion DNA synthesis (TLS) to escape from the risk of UV-induced DNA damage [29, 30]. Very interestingly, apoptotic cell death of *GCN5*$^{-/-}$ cells was appreciably accelerated as compared with that of wild-type DT40 cells [31]. Moreover, *GCN5*$^{-/-}$ cells showed a remarkable sensitivity only to UV-irradiation but not to other DNA-damaging agents. Semiquantitative RT-PCR showed that transcription of the DNA polymerase η (POLH) gene is dramatically down-regulated in *GCN5*$^{-/-}$ cells

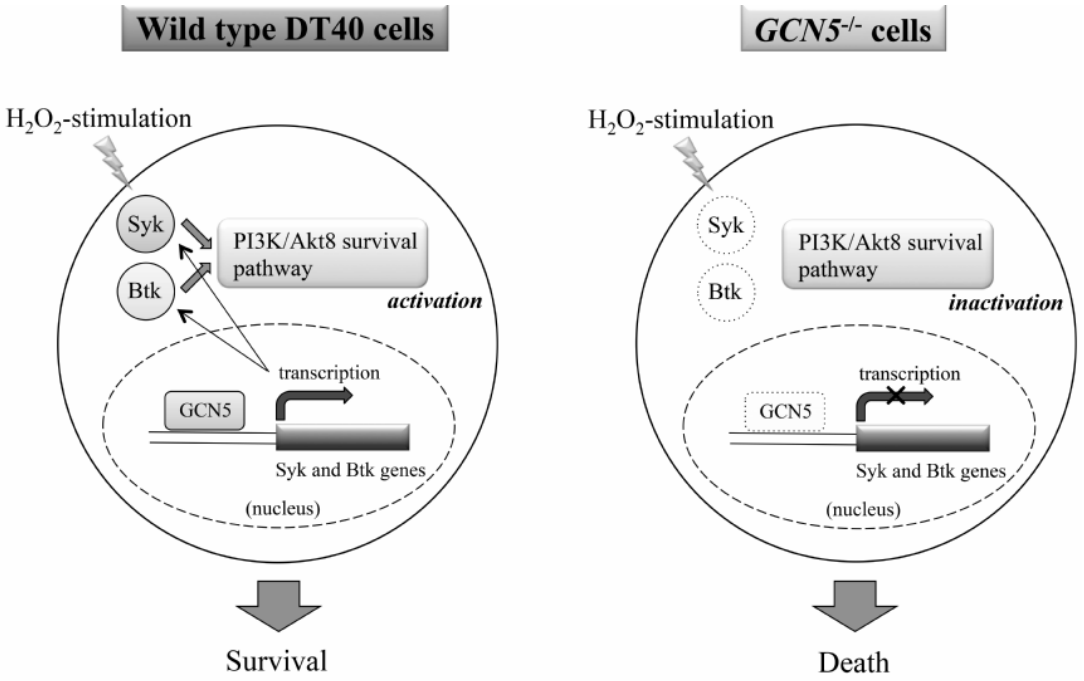

Fig. 4. GCN5 participates in the regulation of response to H_2O_2-induced oxidative stress through controlling gene expressions of Syk and Btk. Semiquantitative RT-PCR showed that gene expressions of Syk and Btk in *GCN5*$^{-/-}$ cells are remarkably decreased as compared with those in DT40 cells. Treatment with H_2O_2 showed that the viability of *GCN5*$^{-/-}$ cells is markedly decreased as compared with that of DT40 cells. ChIP assay revealed that GCN5 binds to proximal 5'-upstream regions of Syk and Btk genes. These results suggest that GCN5 regulates the activation of PI3K/Akt8 survival pathway in B cells exposed to endogenous H_2O_2 through controlling gene expressions of Syk and Btk.

(to ~25%). As is well known, the deficiency of POLH, which is essential for TLS over UV-induced DNA damage such as CPDs and 6-4PDs, is responsible for a variant form of xeroderma pigmentosum [32]. To investigate whether or not the down-regulation of POLH causes the enhanced sensitivity to UV-irradiation in *GCN5*$^{-/-}$ cells, ectopic expression of the POLH gene was performed using human POLH cDNA. As expected, ectopic expression of human POLH cDNA in *GCN5*$^{-/-}$ cells dramatically reversed the sensitivity to UV-irradiation of *GCN5*$^{-/-}$ cells to almost the same level in wild-type DT40 cells. In addition, ChIP assay revealed that GCN5 binds to the 5'-flanking region of the chicken POLH gene, and acetylates H3K9 but not H3K14 *in vivo*. These data suggest that GCN5 takes part in transcriptional regulation of the POLH gene through alterations in the chromatin structure with acetylation of histone H3 surrounding its 5'-flanking region, and protects vertebrate cells against UV-induced DNA damage through controlling POLH gene expression (Fig. 5).

GCN5 regulates B cell differentiation

Normal B cell differentiation in vertebrates requires various specific transcription factors, such as Bach2, Bcl-6, Blimp-1, E2A, EBF1, Ikaros, IRF-4, MITF, OBF-1, Pax5 PU.1, Xbp-1 and others [33-45]. During the differentiation, gene expressions of these transcription factors must be strictly controlled by epigenetic manners including histone acetylation. Semiquantitative RT-PCR and immunoblotting showed that GCN5 deficiency causes drastic decrease in both mRNA and protein levels of Blimp-1 and IRF-4 [46]. Gene expression of Blimp-1 was markedly up-regulated by ectopic expression of IRF-4 in *GCN5*$^{-/-}$ cells, whereas ectopic expression of Blimp-1 in them had an insignificant effect on gene expression of IRF-4. In addition, ChIP assay revealed that GCN5 binds to the IRF-4 gene around its 5'-flanking region

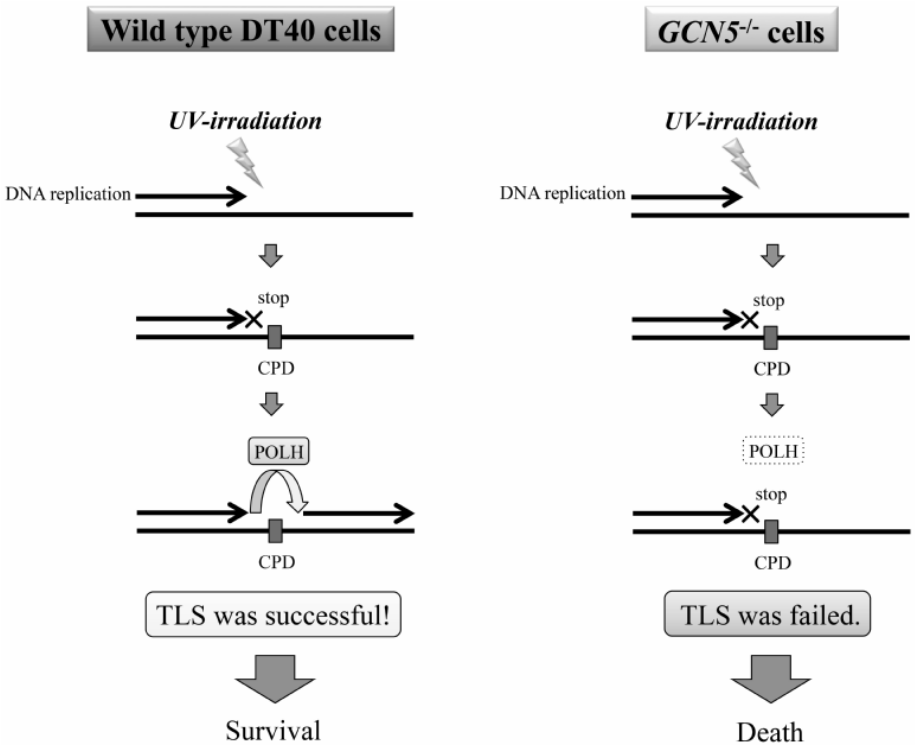

Fig. 5. Failure of POLH-mediated UV-tolerance in *GCN5*$^{-/-}$ cells. Apoptotic cell death of *GCN5*$^{-/-}$ cells was appreciably accelerated as compared with that of DT40 cells, and *GCN5*$^{-/-}$ cells showed remarkable sensitivity to UV-irradiation but not to other DNA-damaging agents. Semiquantitative RT-PCR showed that transcription of DNA polymerase η (POLH) gene is dramatically down-regulated in *GCN5*$^{-/-}$ cells. Deficiency of POLH, which is essential for TLS over UV-induced DNA damage such as CPDs and 6-4PDs, is responsible for a variant form of xeroderma pigmentosum. To investigate whether the down-regulation of POLH causes the enhanced sensitivity to UV-irradiation in *GCN5*$^{-/-}$ cells, ectopic expression of POLH gene was performed using human POLH cDNA. Ectopic expression of human POLH cDNA in *GCN5*$^{-/-}$ cells dramatically reversed the sensitivity to UV-irradiation of *GCN5*$^{-/-}$ cells to almost the same level in DT40 cells. ChIP assay revealed that GCN5 binds to the 5'-flanking region of the POLH gene and acetylates H3K9 but not H3K14. These data suggest that GCN5 takes part in the transcriptional regulation of the POLH gene through alterations in the chromatin structure with acetylation of histone H3 surrounding its 5'-flanking region, and protects vertebrate cells against UV-induced DNA damage through controlling POLH gene expression.

and acetylates H3K9 residues within the chromatin surrounding the region *in vivo*, suggesting that gene expression of IRF-4 is regulated by GCN5. These results suggest that GCN5 is essential for IRF-4 gene expression followed by transactivation of Blimp-1 and plays an important role in epigenetic regulation of B cell differentiation [47].

On the other hand, p300/CBP-associated factor (PCAF) deficiency brought about remarkable decrease in the mRNA levels of Bcl-6 and Pax5, and significant increase in that of Blimp-1 in DT40 cells [48]. ChIP assay showed that PCAF deficiency causes remarkable decrease in acetylation levels of both H3K9 and H3K14 residues within the chromatin surrounding the 5'-flanking regions of Bcl-6 and Pax5 genes *in vivo*, suggesting that their gene expressions are regulated by PCAF. These results suggest that PCAF activates transcriptions of Bcl-6 and Pax5 genes, resulting in down-regulation of Blimp-1 gene expression, and acts as a key factor in epigenetic regulation of B cell development.

After all, GCN5 activates transcription of IRF-4 followed by up-regulation of Blimp-1 gene expression, while PCAF causes transcriptional activation of Bcl-6 and Pax5 genes, resulting in down-regulation of Blimp-1 gene expression. In addition, we showed that GCN5 up-regulates

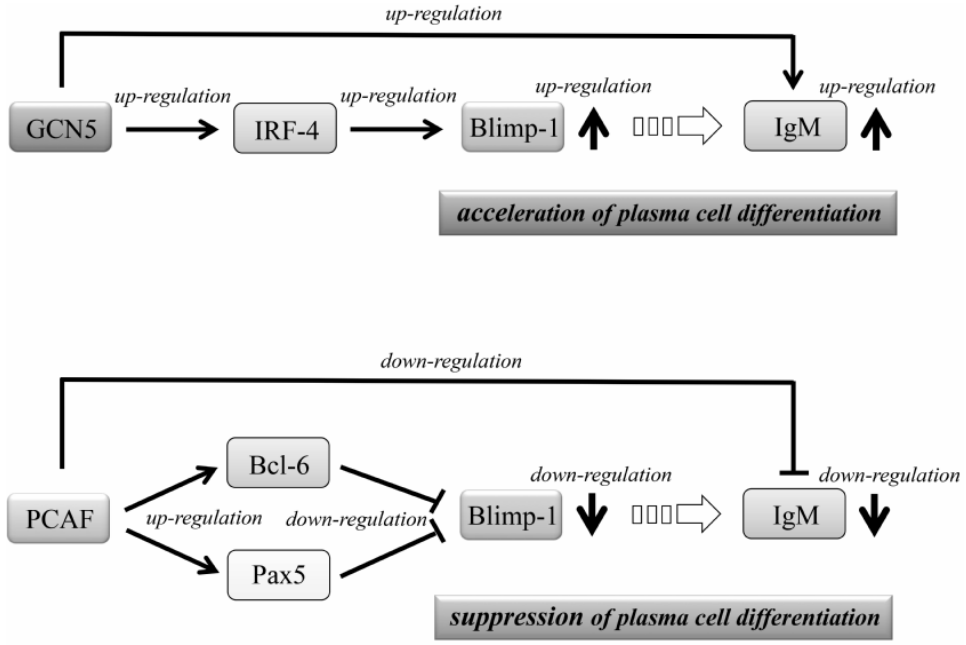

Fig. 6. GCN5 and PCAF participate in the regulation of B cell differentiation through controlling gene expressions of B cell differentiation-related transcription factors and IgM. GCN5 is essential for IRF-4 gene expression followed by transactivation of Blimp-1 and plays an important role in the epigenetic regulation of B cell differentiation. PCAF activates transcriptions of Bcl-6 and Pax5 genes resulting in down-regulation of Blimp-1 gene expression, and acts as a key factor in the epigenetic regulation of B cell development. In addition, GCN5 up-regulates IgM synthesis through activation of IgM heavy chain gene expression, and PCAF down-regulates secretory IgM synthesis through suppression of secretory IgM heavy chain gene expression. These results reveal that GCN5 and PCAF play opposite roles in transcriptional control of Blimp-1 (a master regulator of plasma cell differentiation) and also in the regulation of IgM production in immature B cells.

IgM synthesis through activation of IgM heavy chain gene expression [49] and PCAF down-regulates secretory IgM synthesis through suppression of secretory IgM heavy chain gene expression [50]. These findings revealed that GCN5 and PCAF play opposite roles in transcriptional control of Blimp-1, which is a master regulator of plasma cell differentiation, as well as in the regulation of IgM production in immature B cells (Fig. 6).

GCN5 regulates endoplasmic reticulum (ER) stress-induced apoptosis

As is well known, ER is an important organelle for lipid synthesis, drug metabolism, calcium storage and synthesis of membrane and secretory proteins (and also their translation, folding and maturation) in eukaryotic cells [51-53]. Catastrophe of these ER functions leads to the ER stress, which causes various diseases such as cancer [54], neurodegenerative diseases [55, 56] and diabetes [57]. Prolonged ER stress also brings about apoptosis through activation of various signal transduction pathways [55, 58, 59]. GCN5 deficiency in DT40 cells caused drastic resistance against apoptosis induced by pharmacological ER stress agents such as thapsigargin and tunicamycin [60]. The Bcl-2 family is known as a key factor in the regulation of prolonged ER stress-induced apoptosis [55, 59]. We previously showed that the mRNA level of Bcl-2, a strong anti-apoptotic factor, is drastically increased in *GCN5*$^{-/-}$ cells [11]. In contrast, the mRNA level of Bcl-xL was decreased and that of Bax was not altered in *GCN5*$^{-/-}$ cells. Taken together, there is a possibility that the remarkably increased Bcl-2 could cause the drastic resistance against pharmacological ER stress-induced apoptosis of *GCN5*$^{-/-}$ cells. As expected, pharmaceutical analysis using some specific Bcl-2 inhibitors showed that the drastic resistance against prolonged ER stress-induced apoptosis is due to up-regulation of Bcl-2

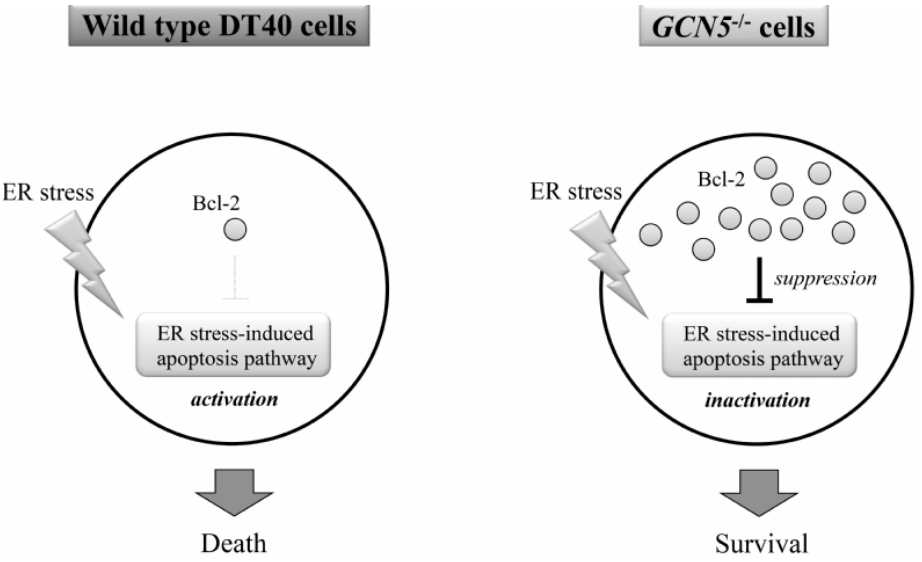

Fig. 7. The elevated Bcl-2 level participates in the suppression of endoplasmic reticulum (ER) stress-induced apoptosis in *GCN5*$^{-/-}$ cells. Prolonged ER stress brings about apoptosis through activation of various signal transduction pathways. GCN5 deficiency in DT40 cells caused drastic resistance against apoptosis induced by pharmacological ER stress agents. The Bcl-2 family is known as a key factor in the regulation of prolonged ER stress-induced apoptosis. In *GCN5*$^{-/-}$ cells, the mRNA level of Bcl-2 (a strong anti-apoptotic factor) was drastically increased, that of Bcl-xL was decreased, and that of Bax was not altered. There is a possibility that the remarkably increased Bcl-2 level could cause the drastic resistance against pharmacological ER stress-induced apoptosis of *GCN5*$^{-/-}$ cells. In addition, some specific Bcl-2 inhibitors showed that the drastic resistance against prolonged ER stress-induced apoptosis is due to up-regulation of Bcl-2 gene expression in *GCN5*$^{-/-}$ cells. These results reveal that GCN5 regulates prolonged ER stress-induced apoptosis through controlling Bcl-2 gene expression.

gene expression in *GCN5*$^{-/-}$ cells. These data reveal that GCN5 regulates prolonged ER stress-induced apoptosis through controlling Bcl-2 gene expression (Fig. 7).

CONCLUSION

As mentioned above, we revealed various inherent physiological functions of GCN5 using gene targeting techniques in DT40 cells derived from chicken immature B cells. First, very interestingly, GCN5 participates in the regulation of various cell death pathways. GCN5 deficiency induced apoptosis [11] and accelerated H_2O_2 [27] and UV-irradiation induced cell death [31], while it suppressed BCR-signaling [17] and ER stress-mediated cell death [60] in DT40 cells. In addition, previous studies showed that loss of GCN5 causes early embryonic lethality [8, 9], neuronal apoptosis [61], and apoptosis of embryonic stem cells [62]. Thus, GCN5 is one of the most powerful supervisors in cell life and death decision mechanisms in vertebrates. Second, GCN5 is a key factor in the regulation of differentiation of leukocytes. GCN5 deficiency brings about loss of O_2^--generating activity in DT40 cells [22]. Further investigations revealed that GCN5 regulates the O_2^--generating system in leukocytes (B cells and monocytes/macrophages) through controlling the NOX2 (gp91-phox) gene expression. Because expression of the O_2^--generating system is a typical marker for mature monocytes/macrophages, GCN5 should be an important factor for their differentiation. On the other hand, GCN5 also regulates negative selection; i.e., the process of eliminating autoreactive B cells [17]. In addition, we showed that in B cells GCN5 is essential for IRF-4 gene expression followed by transactivation of Blimp-1 [46] and up-regulates IgM synthesis through activation of IgM heavy chain gene expression [49]. Moreover, we also revealed that GCN5 up-regulates gene expression of protein kinase C-θ while EBF1, one of the B cell differentiation-related transcription factors, down-regulates it [63]. These results suggest that GCN5 acts as a key factor that cannot be replaced by any other

molecules in epigenetic regulation of terminal differentiation of B cells. In addition, some recent studies showed that GCN5 is required for activation and differentiation of T cells [64, 65]. Based on these findings, we propose that GCN5 should be one of the comprehensive and terminal supervisors in immune system through controlling activation and differentiation of leukocytes (B cells, T cells and monocytes/macrophages). However, very little is known about inherent physiological functions of GCN5 in various other tissues and cells. Therefore, studies on GCN5 will become more important for elucidation of mechanisms of epigenetic regulation of life.

ACKNOWLEDGMENTS

We thank A. Komuku for support in the preparation of the manuscript.

CONFLICT OF INTEREST STATEMENT

There are no conflicts of interest.

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