Biochemical Pharmacology 83 (2012) 296–303

Contents lists available at SciVerse ScienceDirect

Biochemical Pharmacology

journal homepage: www.elsevier.com/locate/biochempharm

Multifunctional Ebselen drug functions through the activation of DNA damage response and alterations in nuclear proteins

Gajendra K. Azad [a], Shah Jaimin Balkrishna [c], Narayanan Sathish [b], Sangit Kumar [c], Raghuvir S. Tomar [a,*]

[a] Laboratory of chromatin Biology, Department of Biology, Indian Institute of Science Education and Research, Bhopal – 462023, India
[b] Department of Biology, Indian Institute of Science Education and Research, Bhopal – 462023, India
[c] Department of Chemistry, Indian Institute of Science Education and Research, Bhopal – 462023, India

ARTICLE INFO

Article history:
Received 11 August 2011
Accepted 12 October 2011
Available online 18 October 2011

Keywords:
Ebselen
DNA damage response
Chromatin
Post translational modifications

ABSTRACT

Several studies have demonstrated that Ebselen is an anti-inflammatory and anti-oxidative agent. Contrary to this, studies have also shown a high degree of cellular toxicity associated with Ebselen usage, the underlying mechanism of which remains less understood. In this study we have attempted to identify a possible molecular mechanism behind the above by investigating the effects of Ebselen on *Saccharomyces cerevisiae*. Significant growth arrest was documented in yeast cells exposed to Ebselen similar to that seen in presence of DNA damaging agents (including methyl methane sulfonate [MMS] and hydroxy urea [HU]). Furthermore, mutations in specific lysine residues in the histone H3 tail (H3 K56R) resulted in increased sensitivity of yeast cells to Ebselen presumably due to alterations in post-translational modifications of histone proteins towards regulating replication and DNA damage repair. Our findings suggest that Ebselen functions through activation of DNA damage response, alterations in histone modifications, activation of checkpoint kinase pathway and derepression of ribonucleotide reductases (DNA repair genes) which to the best of our knowledge is being reported for the first time. Interestingly subsequent to Ebselen exposure there were changes in global yeast protein expression and specific histone modifications, identification of which is expected to reveal a fundamental cellular mechanism underlying the action of Ebselen. Taken together these observations will help to redesign Ebselen-based therapy in clinical trials.

1. Introduction

Ebselen is an anti-oxidant organo-selenium compound with immunomodulatory effects [1]. It has been described as a cytoprotective [2,3], cardioprotective [4] and neuroprotective [5,6] agent with anti-inflammatory therapeutic properties [7]. Ebselen has also been commonly employed in clinical trials as an antistroke medication. Interestingly Ebselen also possesses anti-inhibitory effects against apoptosis induced by free radicals. Despite it is reported anti-oxidative [8] and anti-inflammatory effects [1,9], high concentration of Ebselen have been shown to be toxic to cells. This stands exemplified in various studies, including one by Yang et al. [10] and in another report wherein Ebselen was shown to induce apoptosis or cell death of $HepG_2$ cells and hybridoma cells respectively. Additionally, inhibitory effects exerted by Ebselen on lactate dehydrogenase activity in mammals have also been documented in a recent in vivo study [11]. Ebselen is also known to act as a glutathione peroxidase, thus serving as an excellent scavenger of peroxynitrite [12–14]. The above glutathione peroxidase-like activity of Ebselen mediates inhibition of several enzymes including cyclooxygenase and lipoxygenases at micromolar concentrations [15,16]. From the above observations it does become evident that despite being a potential therapeutic drug employed in the treatment of a wide variety of disease symptoms, Ebselen also impart a lot of toxic effects on the cells. There exists currently a lacuna in comprehending the mechanisms underlying the toxicity induced by Ebselen, which definitely mandates bridging.

A diverse range of pharmacological drugs available in the market are known to function by modulating host epigenetics, through activation of the cell cycle checkpoint and DNA repair processes involving transcriptional activation of repair genes ensuring genetic integrity [17–19]. Host epigenetics encompasses the study of non-DNA sequence-related heredity and plays a very critical role in modern medicine due to its potential to delineate the relationship between an individual's genetic background, the environment, aging, and disease processes. Molecular phenomena involving epigenetic changes do occur on the chromatin, the DNA complex, DNA associated histone proteins as well as on other proteins constituting the physiological form of the genome. The epigenome is dynamic and is responsive to these environmental

* Corresponding author. Tel.: +91 755 4092307; fax: +91 755 4092392.
E-mail address: rst@iiserbhopal.ac.in (R.S. Tomar).

0006-2952/$ – see front matter © 2011 Elsevier Inc. All rights reserved.
doi:10.1016/j.bcp.2011.10.011

signals throughout life. It is thus becoming increasingly apparent that many chemical agents trigger changes in gene expression that persist even long after exposure has ceased [20–37].

Of significant concern is the fact, that commonly used pharmaceutical drugs do have the potential to induce persistent epigenetic changes associated with higher dosage of drugs [38,39]. These epigenetic changes influence signalling pathways culminating in alterations of transcription factor activity at their respective gene promoters. These alterations result in pleuripotent effects including altered expression levels of cell surface receptors, signalling molecules, and other proteins essential towards altering genetic regulatory circuits [20,40–45]. Chronic exposure of cells to pharmaceutical drugs, results in their adaptation (through a hitherto unknown hypothetical process) to the source only resulting in more permanent modifications to DNA methylation and chromatin structure, leading to the total alteration of a given epigenetic network [46–48]. A probable mechanism by which the drugs (including Ebselen) exert toxicity (typified as cell growth arrest) is through modifications in the host cell epigenetic network. This is of significance given the fact that epigenetic/post-translational modifications of N-terminal regions of histones influence multiple cellular processes including replication, transcription, DNA repair, recombination and chromatin assembly.

Given this background and the fact that the mechanisms underlying Ebselen action is not known (especially in relation to the host epigenetic network), we had initiated this study to comprehend the pathways governing the action of Ebselen. Towards this, we have used wild type and histone tail mutants of yeast strains and treated them with either known DNA-damaging agents like methyl methanesulphonate (MMS) or with varying concentration of Ebselen to analyze their growth phenotype. Surprisingly cells treated with Ebselen exhibited phenotypes similar to the phenotypes observed subsequent to MMS and hydroxyurea treatments, suggesting that Ebselen induced phenotypes could also be due to the activation of DNA damage response pathway. We analyzed the expression of highly inducible ribonucleotide reductases (DNA repair genes) upon treatment with Ebselen. We found that Ebselen activates the expression of these genes similar to the expression observed upon treatment with the known DNA damaging agent methyl methane sulfonate. We also checked the level of DNA damage checkpoint kinase target protein Sml1, which is known to be degraded upon DNA damage. Interestingly we found degradation of Sml1 upon Ebselen treatment. Further investigations have revealed that Ebselen functions through the epigenetic modifications of histones (specifically histone tails). Our findings also suggest that the morphological changes in yeast cells, which we have observed upon Ebselen treatment could serve as a useful indicator in helping to decide the suitable dose of this drug towards effective treatment of disease symptoms without drastically impacting the normal cellular function.

2. Materials and methods

2.1. Reagents and yeast strains

All the chemical reagents used in this study were purchased from Sigma–Aldrich India, Merck India, Thermo Scientific India, GE Healthcare India, Invitrogen India and HiMedia India. DNA damaging agents including MMS and hydroxyurea (HU) were purchased from Sigma–Aldrich, India. Ebselen was made in our laboratory as described and published previously [49]. Ebselen was dissolved in 100% of DMSO and was used to treat the yeast cells in liquid as well as solid medium.

2.2. Electrophoresis and Western blotting

Whole cell extracts were made from Ebselen treated cells and were resolved on SDS-10% PAGE gels, transferred on to nitro cellulose membrane and subjected to western blot analysis. Western blotting was carried by following the standard protocol. Blotting onto nitrocellulose membrane was carried out in transfer buffer (25 mM Tris, 192 mM glycine (pH 8.3, 10% methanol and 0.01% SDS) overnight at 50 mA, 4 °C using a BioRad wet transfer apparatus. Blocking was done in blocking solution (2.5% NFDM in 10 mM Tris–HCl, pH 7.5, 150 mM NaCl, 0.1% Tween 20) for 30 min. Primary and secondary antibody incubations were carried out for 1 h. To study epigenetic changes upon Ebselen treatment, following antibodies were used: H3K4me1 (Abcam, 8895), H3K4me2 (Abcam, 32356), H3K27me2 (Abcam, 24684), H3 K27ac (Abcam, 45173), H3K23ac (Abcam, 46982), H3K9Ac (Abcam 69830), H4K16ac (Abcam, 61240), H4K8ac (Abcam, 15823). To study expression of DNA Damage response genes upon Ebselen treatment following antibody were used; RNR1 (Agrisera, ASO9 576), RNR2 (Agrisera, ASO9 575), SML1 (Agrisera, AS10 847) and for control GAPDH (Abcam, 37168). Signals were detected by chemiluminescence using Fuji gel-dock system. HRP conjugated secondary antibodies and ECL reagents used were purchased from GE Healthcare. Dilutions of antibodies were used as per the instructions provided by the manufacturers.

The yeast strains employed in this study were all laboratory strains and are listed in Table 1.

2.3. Growth test

To investigate the effect of Ebselen on the growth of yeast cells, wild-type *Saccharomyces cerevisiae* yeast strains were inoculated into YPD (1% yeast extract, 2% peptone, and 2% dextrose) liquid medium and grown to saturation by incubation of the cultures at 30 °C (with shaking). About 3 μl of the yeast saturated cultures were serially diluted (10^{-1}, 10^{-2} and 10^{-3}) in 1.0 ml of sterile media (YPD). 2–3 μl of each undiluted and diluted culture were spotted onto plates containing either solid standard YPD medium

Table 1
List of strains used in this study.

Strain	Genotype
W1588-4c	MATa *ade2-1 can1-100 his3-11,15 leu2-3,112 trp1-1 ura3-1 RAD5+*
CLY460	MATα *ura3-52 leu2-3,112 trp1-289 his3Δ1 Δ(hhf1-hht1)(hhf2-hht2)* pCL460[CEN TRP1 HHF1 hht1-3 H3(K4,9,14,18,23,27Q)]
MSY 421 H3K56R	MATa *!(hht1-hhf1) !(hht2-hhf2) leu2-3,112 ura3-62 trp1 his3 p*(TRP1,CEN,hht2-HHF2) hht2 K56R
PY015	Isogenic to PY014; carries pMP012 (CEN6 TRP1 hta1Δ4–20 HTB1), Histone H2A Δ(4–20)
PY018	Isogenic to PY014; carries pMP023 (CEN6 TRP1 hta1-K4,7G HTB1), Histone H2A K4, 7G
PY052	Isogenic to PY014; carries pMP074 (CEN6 TRP1 hta1 Δ4–8 HTB1), Histone H2A Δ(4–8)
PY054	Isogenic to PY014; carries pMP076 (CEN6 TRP1 hta1Δ12–20 HTB1), Histone H2A Δ(12–20)
PY055	Isogenic to PY014; carries pMP077 (CEN6 TRP1 hta1Δ16–20 HTB1), Histone H2A Δ(16–20)
PY058	Isogenic to PY014; carries pMP080 (CEN6 TRP1 hta1 S19A HTB1), Histone H2A S19A
PY014	WT,MATa*ade2-1 trp1-1 can1-100 leu2-3,112 his3-11,15 ura3 hta1-htb1*::HIS3 hta2 htb2::LEU2 plus pMP002 (CEN6 TRP1 HTA1 HTB1)

or YPD medium incorporated with increasing concentrations of Ebselen (in the range of 10–100 μM). Plates were incubated at 30 °C and growth of the yeast strains were recorded at periodic time intervals of 24, 48 and 72 h by scanning the plates using HP scanner. Yeast cultures obtained by growth of yeast cells in YPD broth incorporated with DNA-damaging agents (0.01% MMS or 100 mM HU) served as controls. These yeast cultures too were serially diluted (as above) and plated onto plates containing solid standard YPD medium and growth patterns were recorded following incubation of plates at 30 °C.

To assess the effect of Ebselen on the yeast cell epigenetics, both the wild-type yeast strain and the histone-tail mutants (with amino acid deletions/substitutions in H2A and amino acid substitutions in H3 enlisted in Table 1) were grown to saturation at 30 °C (with shaking) in regular YPD medium. These cultures were serially diluted and (along with undiluted cultures) were spotted onto YPD plates incorporated with increasing concentrations of Ebselen (10–100 μM) and photographs of the yeast growth was taken post 48 h incubation of the plates at 30 °C.

2.4. Microscopy

Wild type yeast strains were grown in regular YPD liquid medium as above to an absorbance (OD_{600}) of 0.8. These yeast cultures were either left untreated or were treated with 0.1% DMSO (controls) or were treated with 100 μM Ebselen. At every 30 min interval (upto a time point of 120 min) subsequent to the above treatment, small aliquots of the yeast cultures were placed onto slides and were visualized under a LEICA DM500 microscope (installed with the LAS EZ-V1.7.0 software) to record the morphology of the yeast cells.

2.5. Preparation of protein extracts and electrophoresis

The wild-type yeast strains were inoculated into 250 ml of regular YPD medium and the cultures were incubated at 30 °C (with shaking) to reach an absorbance (OD_{600}) of about 1.0. An aliquot of these yeast cultures (40 ml) was kept aside as an untreated control. Ebselen was subsequently added to the remaining yeast culture (to a final concentration of 100 μM) and 40 ml aliquots as above were taken out at every 5 min interval (5, 10, 15, 20 and 25 min) subsequent to Ebselen treatment. The yeast cells thus obtained from both the Ebselen treated and untreated cultures were washed with water and the cell pellets were subsequently stored at −80 °C till protein extraction. Protein extracts were obtained from the frozen yeast cell pellets with 20% trichloro acetic acid (TCA) by following a standard protocol.

The protein extracts were mixed with equal volume of 2X SDS-PAGE sample loading buffer (100 mM Tris–HCl (pH 6.8), 200 mM DTT, 4% SDS, 0.2% bromophenol blue and 20% glycerol), boiled for 5 min and resolved by electrophoresis on a SDS-12% polyacrylamide gel. Following electrophoresis the gel was stained with 0.1%

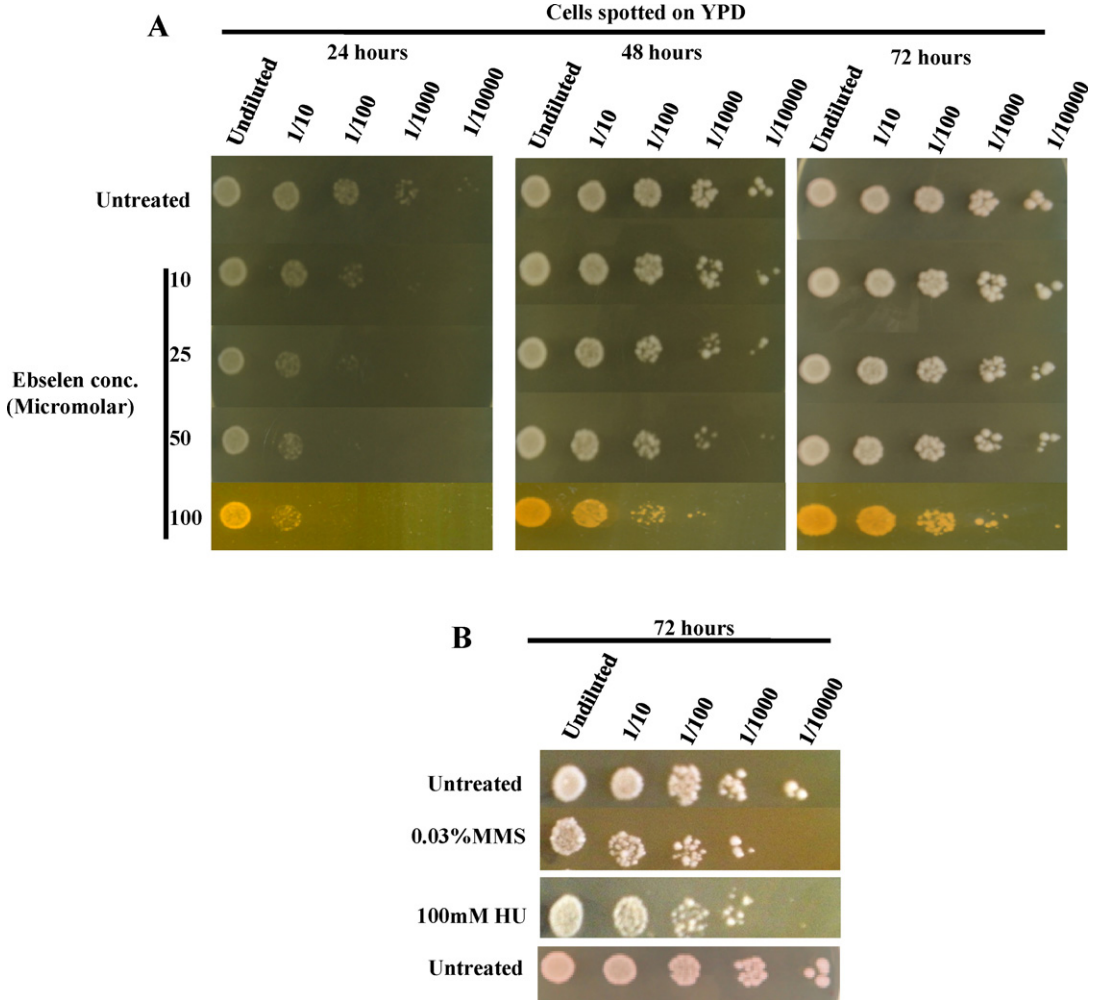

Fig. 1. Effects of Ebselen on growth of yeast cells are similar to the effects of MMS and HU that causes DNA double strand break. A. Spot test for cells grown in presence of Ebselen. Yeast strain-WT1588 W303 was spotted on YPD plates containing Ebselen in the range of 10–100 μM concentrations as indicated in the figure. B. Growth test in presence of MMS and Hydroxyurea incubated for 72 h.

of Coomassie Brilliant Blue stain, destained with 10% solution of methanol and acetic acid and photographed.

3. Results

3.1. Examination of growth phenotype of yeast cells in the presence of Ebselen

To examine the growth phenotype of wild-type (WT) yeast cells in the presence of increasing concentrations of Ebselen, yeast cells were grown to a saturated phase (OD_{600} 2–3) and serial dilutions made. A 3 μL of both the diluted and undiluted yeast cultures in 1.0 ml of sterile media (YPD) were sequentially spotted on a series of plates containing YPD solid medium incorporated with increasing concentrations of Ebselen (in the range of 10–100 μM). The yeast culture spotted YPD plates were incubated at 30 °C for upto 72 h and yeast growth was monitored at 24 h intervals. Ebselen was found highly toxic to the yeast cells as shown by the drastic reduction in the growth of the yeast cells with increasing concentration of the drug (Fig. 1A). Interestingly these reductions were very comparable with that witnessed following growth of yeast cells in YPD plates incorporated with known DNA damaging agents such as Methyl methane sulfonate (0.01%) or Hydroxyurea (100 mM) (Fig. 1B). Subsequently we examined the morphology of Ebselen treated yeast cells under a LEICA DM500 microscope. Towards this, yeast cells grown in regular YPD medium (incorporated with 100 μM Ebselen) were photographed at different time points (as indicated in Fig. 2). A yeast cell culture in YPD medium (incorporated with 0.1% DMSO) along with a yeast culture (in plain YPD medium) were included as controls. With increasing time of exposure to Ebselen, yeast cells exhibited characteristic morphological changes including shrinkage in cell sizes as compared to the controls combined with an obvious reduction in the number of yeast cells (Fig. 2). This to the best of our knowledge represents the first documented evidence of the growth inhibitory effects of Ebselen.

3.2. Ebselen treatment induces alterations in protein expression/modification

Several reports have indicated the anti-oxidatory potential of Ebselen in imparting protection against diseases at low concentrations [50]. However the effects of Ebselen at higher concentrations on both protein expression and DNA integrity have rather remained unclear. Towards analyzing the effects of Ebselen on yeast cytosolic and nuclear proteins, we prepared whole cell extracts from yeast cells treated with 100 μM of Ebselen for the specified time points (as indicated in Fig. 3) and analyzed them by SDS-10% polyacrylamide denaturing gel electrophoresis. Interestingly, a protein band with an approximate molecular weight of about 40 kDa detected in yeast whole cell extracts obtained from cells treated with Ebselen (Lane 1, Fig. 3) was repositioned to a slightly higher molecular weight position (lane 2) following exposure of yeast cells to 100 μM Ebselen at different time points (Lanes 2–6, Fig. 3A). It is tempting to speculate that there could be a possibility for the above interesting phenomenon. The post-translational modification such as phosphorylation of the 40 kDa protein band shown by an arrow in Fig. 3A in 'lane 1' can cause a shift of this protein in SDS-PAGE gel (compare lane 1 with lane 2 of Fig. 3A). Identification of the target 40 kDa protein and its presumable post-translational modification (resulting in shifting of band size) is currently under extensive investigation in our laboratory. This is expected to reveal a fundamental cellular mechanism underlying the action of Ebselen. We then also analyzed the integrity of the genomic DNA upon treatment (Fig. 3B). We observed the fragmentation of genomic DNA upon

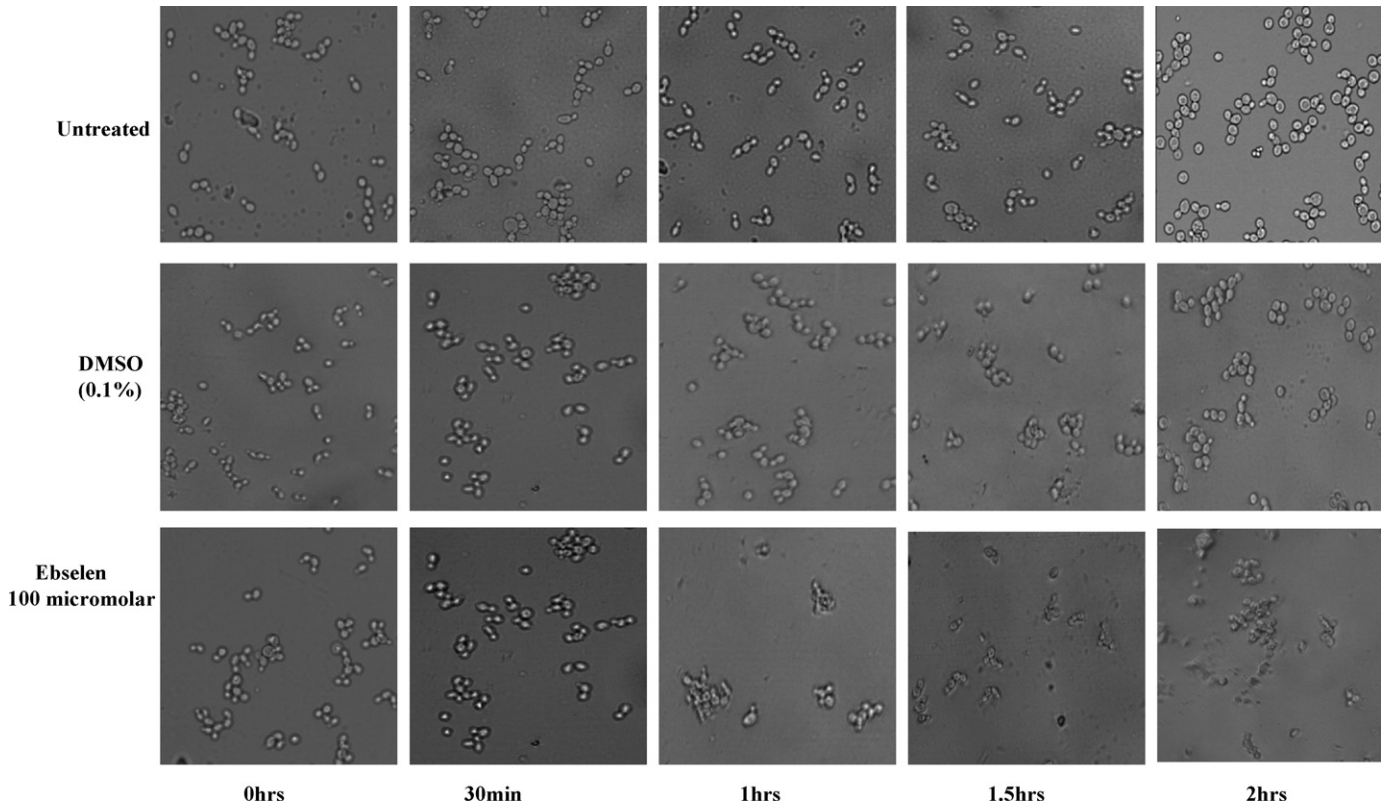

Fig. 2. Ebselen treatment causes shrinkage of cells. Yeast cells were grown in regular YPD medium in presence of 100 μM concentration of Ebselen, incubated for time points indicated and photographed.

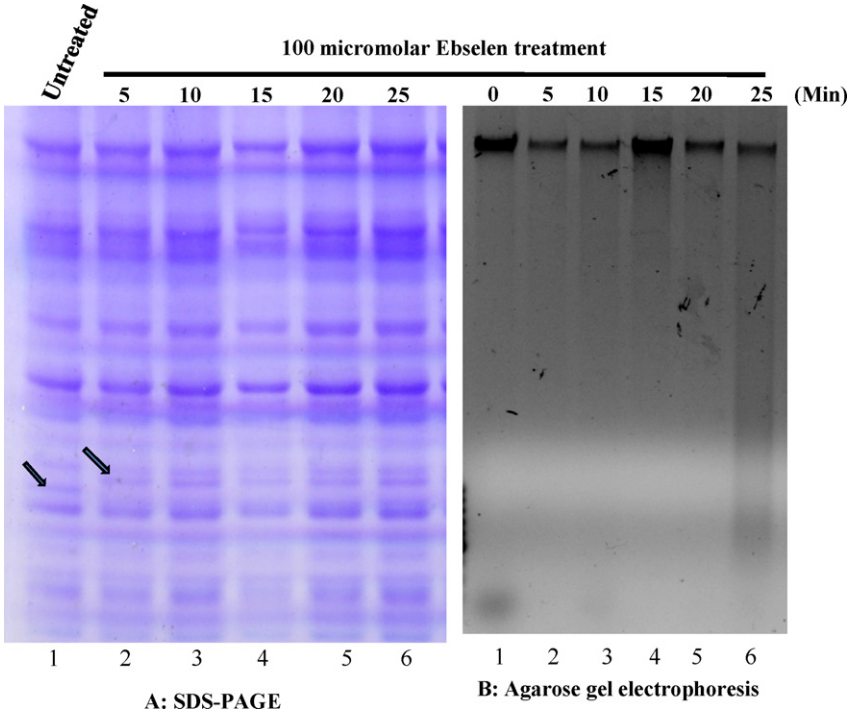

Fig. 3. Ebselen causes alterations in proteins and DNA: A. SDS-10% polyacrylamide gel electrophoresis for the analysis of proteins extracted from Ebselen treated yeast cells. Arrow indicates change in protein expression upon treatment with 100 μM concentration of Ebselen. B. Genomic DNA was isolated from Ebselen treated cells and analyzed on 1.5% agarose gel electrophoresis in Tris Acetic Acid EDTA (TAE) buffer.

Ebselen treatment. For the analysis of changes in post-translational modifications in nuclear histone proteins H3 and H4 upon Ebselen treatment, we did Western blotting (Fig. 4). To our surprise mono and dimethylation of H3 Lysine 4 has decreased drastically upon treatment with the Ebselen suggesting that Ebselen function through alterations in post-translational modifications of histone proteins.

3.3. *Ebselen function through the modulation of histone modifications and activation of DNA damage response pathway*

Finally we were motivated to identify a possible mechanism behind the Ebselen-induced alterations in yeast cellular morphology and protein expression. We hypothesized a probable mechanism through which Ebselen exerts its toxic effect on the yeast cell growth could lie in its ability to mediate post-translational modifications of histone tails given its propensity towards altering cellular epigenetic marks. Towards this we employed yeast strains (harbouring mutations in the histone tails, including strains with amino acid deletions/substitutions in H2A and amino acid substitutions in H3) (Fig. 5) grown to saturation in regular YPD medium. These cultures were serially diluted and (along with undiluted cultures) were spotted onto YPD plates containing increasing concentrations of Ebselen (10–100 μM) and photographs of the yeast growth taken post 48 h incubation at 30 °C.

Interestingly, as compared to the wild-type control, growth patterns of the yeast strain MSY421 (harbouring an amino acid mutation in H3/K56R) alone was noticeably inhibited with increasing concentrations of Ebselen (Fig. 5). Transient modifications of tails of histone proteins including acetylation of lysine 56 on histone H3 (H3-K56) have been implicated in regulation of DNA damage response. Given the finding here that the yeast strain MSY421 (with alterations in H3-K56 acetylation) displayed increased sensitivity towards Ebselen akin to the sensitivity of this strain to other DNA damaging agents like MMS and HU, it is reasonable to suggest that Ebselen probably functions through modulation of epigenetic histone modifications. To confirm our hypothesis further that Ebselen activates DNA damage response, we analyzed the expression of highly inducible DNA damage response enzymes (ribonucleotide reductases, RNR) RNR1 and RNR2 (Fig. 6). We find increased expression of these proteins upon treatment of cells with the Ebselen. We also observed drastic decrease in the level of Sml1 (inhibitor of ribonucleotide reductases) upon Ebselen treatment suggesting that Ebselen may function through activation of DNA damage response pathway.

4. Discussion

Ebselen an organo-selenium compound widely employed in clinical trials is known to possess anti-oxidant properties [51,52]. Through interaction with specific cysteine residues, Ebselen functions as an inhibitor of a diverse group of enzymes including mammalian lipoxygenases, glutathione S-transferase and papain. Despite these well documented observations, the molecular mechanism behind the action of Ebselen has remained unclear. From the aforementioned observations it does become clear that Ebselen similar to a wide range of pharmacological drugs available in the market possesses the potential to exert toxic side effects. Exposure of budding yeast cells to DNA damaging agents (including MMS and HU) triggers breaks in the dsDNA, which in turn induce cell cycle arrest and transcriptional induction of DNA repair genes. In fact in our study, we did observed growth arrest of yeast cells and de-repression (Fig. 6) of DNA repair enzymes following their exposure to Ebselen indicating its modality of action to be similar to that of other DNA damaging agents.

Given the fact that a probable mechanism by which pharmacological drugs exert toxicity (typified as cell growth arrest) is through modifications in the host cell epigenetic network, we were motivated to delineate the possible occurrence of a similar

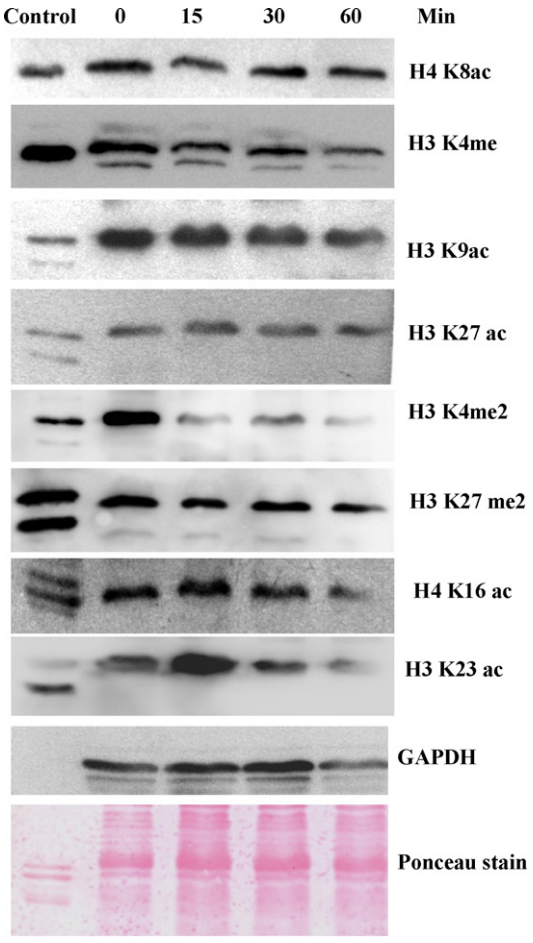

Fig. 4. Ebselen causes alterations in epigenetic modifications in histone proteins: A. Wild type cells were grown in normal YPD medium in presence of 100 μM of Ebselen for different time points as indicated in the figure. Whole cell extracts were made and western blotting was conducted to detect the changes in post-translational modifications in histone H3 and H4. In control lane, purified core histones from chicken liver are loaded. Antibodies used for western blotting are indicated on right side of the figure.

scenario following exposure of cells to Ebselen too. It is well known that the host epigenetic network encompasses the chromatin, the DNA complex, DNA associated histone proteins and other proteins constituting the physiological form of the genome. Interestingly studies focusing on the effects of drugs on the host cell epigenetics have mostly focused on DNA methylation, as a result of which their possible effects on histone modifications has remained largely ignored. Histones are known to exert pivotal roles aimed at regulating the structure and function of transcription genes. Certain epigenetic modifications of histone proteins have been shown to be required for the proper regulation of multiple cellular processes including replication, transcription, DNA repair, recombination and chromatin assembly. A majority of the epigenetic post-translational modifications (including acetylation, methylation, etc.) are known to be localized to the tail extensions of the histones (histone tails). Given the significance of histones and their specific tail domains in epigenetic modulations, it became essential for us to decipher the role of the histone tails in delineating the mechanism by which Ebselen leaves its epigenetic mark.

Indeed, mutations in specific lysine residues in the histone H3 and H2A tail resulted in increased sensitivity of yeast cells to Ebselen (H3K56R mutation). This finding is of significance as acetylation of lysine 56 on histone H3 (H3-K56) has been implicated in regulating replication and is a process that also occurs during DNA damage repair [53]. In fact cells with alterations in H3-K56 acetylation display increased sensitivity towards certain DNA damaging agents [53,54]. A similar such appreciable susceptibility of yeast cells (with the H3K56R mutation) to Ebselen (Fig. 5) and the de-repression of DNA repair genes, ribonucleotide reductases – RNR, (Fig. 6) helps to suggest that Ebselen may also function as a DNA-damaging agent through modulation of histone modifications, which to the best of our knowledge has been reported for the first time. Treatment of cells with Ebselen causes the de-repression of the DNA repair enzymes (Fig. 6) probably through the activation of Mec1-Rad53-Dun1 kinase pathway. It is known from several studies that DNA damaging agents activate checkpoint kinase pathway, which causes the de-repression of RNR genes [55,56]. Activated kinase pathway phosphorylates SML1 [57–59] (inhibitor of RNRs) for the proteasomal degradation of this protein. We have observed drastic decrease in the level of Sml1

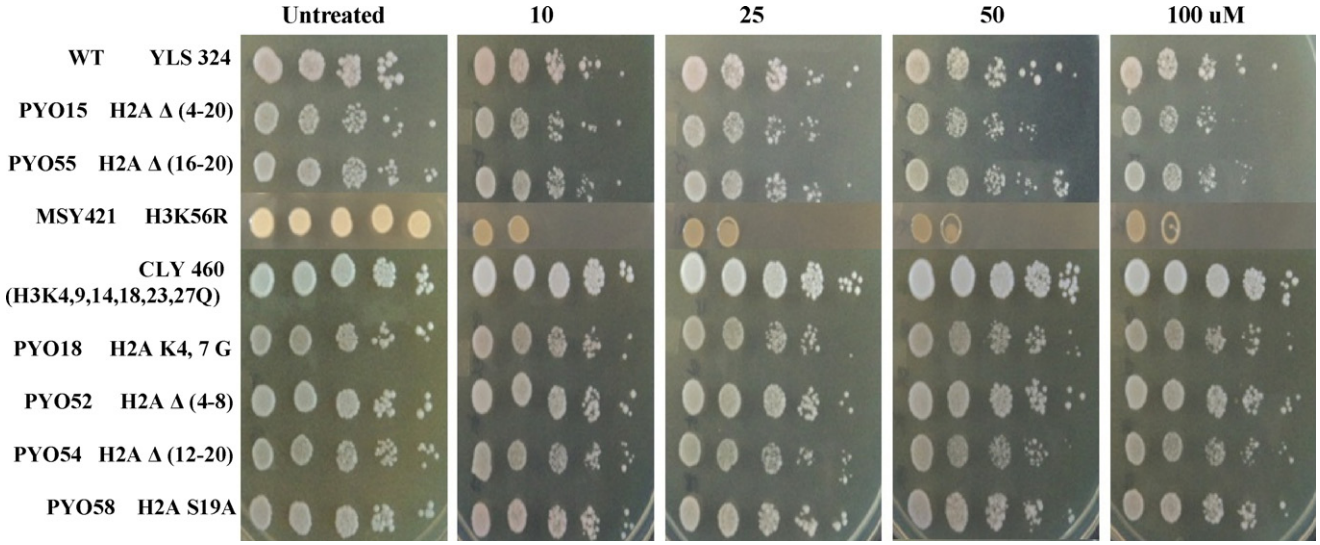

Fig. 5. Tails of histones regulate function of Ebselen: Yeast strains having mutations in histone H2A and H3 as indicated in figure were grown in regular YPD medium to saturation. Cells were serially diluted and spotted on YPD plates containing concentrations of Ebselen in the range of 10–100 μM, incubated at 30 °C temperature and photographed after 48 h.

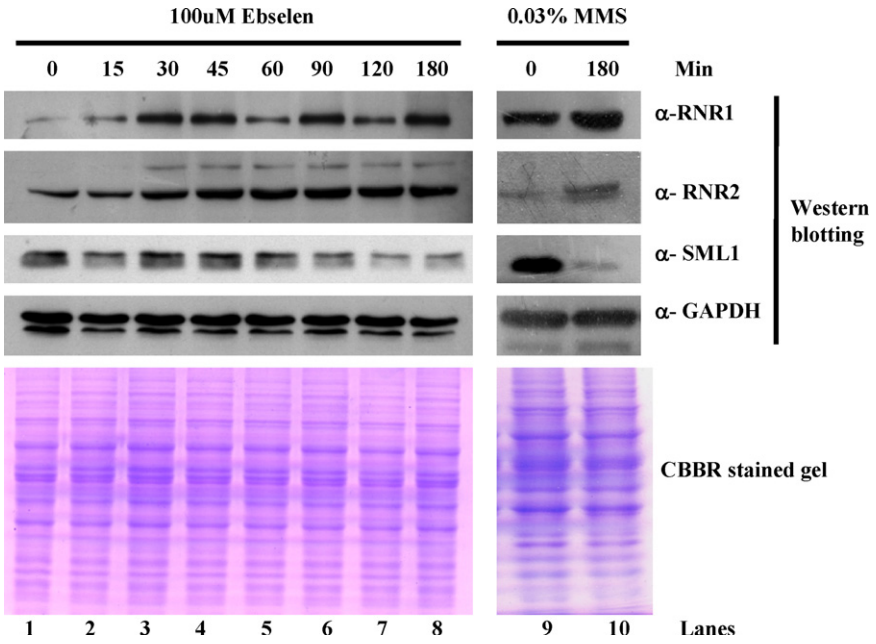

Fig. 6. Ebselen activates DNA damage response through checkpoint kinase pathway: WT yeast strain was grown in normal YPD medium. When OD reached 0.8, Ebselen (100uM final concentration) and 0.03% MMS was added and cells were harvested at indicated time points mentioned in minutes. Whole Cell Extracts (WCE) were made in SDS-PAGE load dye and western blotting was conducted to check the expression of DNA repair enzymes (RNR1 and RNR2) and the inhibitor (SML1) of RNR enzymes upon Ebselen as well as Methyl methane sulfonate (MMS) treatments. GAPDH western blotting and Coomassie blue staining was done for the loading control.

proteins probably due to proteasomal degradation upon Ebselen treatment, which further confirms our hypothesis that Ebselen activates DNA damage response.

Another interesting finding was the changes in expression of some proteins (including a protein with molecular weight 40 kDa) subsequent to Ebselen treatment. Identification of the above and possible other proteins (whose expression is also altered) is expected to reveal a fundamental cellular mechanism underlying the action of Ebselen. An ideal approach towards this would be performance of a comparative global proteomic screen of yeast cells following exposure to Ebselen, which should help identify the candidate protein/s (including possible DNA repair genes) whose expression is altered following the drug treatment. This combined with identification of possible additional histone epigenetic modifications if any subsequent to Ebselen exposure should definitely provide more valuable insights into the mechanisms underlying the action of Ebselen. Outcome of these studies may thus be translated to redesign the Ebselen-based therapy in clinical trials.

Acknowledgements

We thank John Wyrick, Jessica Tyler, Randall H. Morse and David Shore for providing yeast strains. We thank Director of IISER Bhopal for providing infrastructure facility and funds for this study, and all the members of the laboratory for helpful discussions. Council of Scientific and Industrial Research (CSIR) is acknowledged for fellowship support to GKA.

References

[1] Belvisi MG, Haddad EB, Battram C, Birrell M, Foster M, Webber S. Anti-inflammatory properties of Ebselen in a model of sephadex-induced lung inflammation. Eur Respir J 2000;15(3):579–81.
[2] Yang CF, Shen HM, Ong CN. Protective effect of Ebselen against hydrogen peroxide-induced cytotoxicity and DNA damage in HepG2 cells. Biochem Pharmacol 1999;57(3):273–9.
[3] Yang CF, Liu J, Shen HM, Ong CN. Protective effect of Ebselen on aflatoxin B1-induced cytotoxicity in primary rat hepatocytes. Pharmacol Toxicol 2000;86(4):156–61.
[4] Hoshida S, Aoki K, Nishida M, Yamashita N, Igarashi J, Hori M, et al. Effects of preconditioning with Ebselen on glutathione metabolism and stress protein expression. J Pharmacol Exp Ther 1997;281(3):1471–5.
[5] Saito I, Asano T, Sano K, Takakura K, Abe H, Yoshimoto T, et al. Neuroprotective effect of an antioxidant, Ebselen, in patients with delayed neurological deficits after aneurysmal subarachnoid hemorrhage. Neurosurgery 1998;42(2):269–77 (discussion 77–78).
[6] Yamagata K, Ichinose S, Miyashita A, Tagami M. Protective effects of Ebselen, a seleno-organic antioxidant on neurodegeneration induced by hypoxia and reperfusion in stroke-prone spontaneously hypertensive rat. Neuroscience 2008;153(2):428–35.
[7] Posser T, Kaster MP, Barauna SC, Rocha JB, Rodrigues AL, Leal RB. Antidepressant-like effect of the organoselenium compound Ebselen in mice: evidence for the involvement of the monoaminergic system. Eur J Pharmacol 2009;602(1):85–91.
[8] Ozaki M, Nakamura M, Teraoka S, Ota K. Ebselen, a novel anti-oxidant compound, protects the rat liver from ischemia-reperfusion injury. Transpl Int 1997;10(2):96–102.
[9] Beil W, Staar U, Sewing KF. Interaction of the anti-inflammatory seleno-organic compound Ebselen with acid secretion in isolated parietal cells and gastric H+/K(+)-ATPase. Biochem Pharmacol 1990;40(9):1997–2003.
[10] Yang CF, Shen HM, Ong CN. Ebselen induces apoptosis in HepG(2) cells through rapid depletion of intracellular thiols. Arch Biochem Biophys 2000;374(2):142–52.
[11] Lugokenski TH, Muller LG, Taube PS, Rocha JB, Pereira ME. Inhibitory effect of Ebselen on lactate dehydrogenase activity from mammals: a comparative study with diphenyl diselenide and diphenyl ditelluride. Drug Chem Toxicol 2011;34(1):66–76.
[12] Parnham MJ, Kindt S. A novel biologically active seleno-organic compound – III. Effects of PZ 51 (Ebselen) on glutathione peroxidase and secretory activities of mouse macrophages. Biochem Pharmacol 1984;33(20):3247–50.
[13] Batna A, Fuchs C, Spiteller G. Lipid peroxidation in presence of Ebselen. Chem Phys Lipids 1997;87(2):149–58.
[14] Aruoma OI. Scavenging of hypochlorous acid by carvedilol and Ebselen in vitro. Gen Pharmacol 1997;28(2):269–72.
[15] Muller A, Cadenas E, Graf P, Sies H. A novel biologically active seleno-organic compound – I. Glutathione peroxidase-like activity in vitro and antioxidant capacity of PZ 51 (Ebselen). Biochem Pharmacol 1984;33(20):3235–9.
[16] Nakamura Y, Feng Q, Kumagai T, Torikai K, Ohigashi H, Osawa T, et al. Ebselen, a glutathione peroxidase mimetic seleno-organic compound, as a multifunctional antioxidant. Implication for inflammation-associated carcinogenesis. J Biol Chem 2002;277(4):2687–94.
[17] Li A, Eirin-Lopez JM, Ausio J. H2AX: tailoring histone H2A for chromatin-dependent genomic integrity. Biochem Cell Biol 2005;83(4):505–15.

[18] Featherstone C, Jackson SP. DNA-dependent protein kinase gets a break: its role in repairing DNA and maintaining genomic integrity. Br J Cancer 1999;80(Suppl. 1): 14–9.
[19] Hartwell L, Weinert T, Kadyk L, Garvik B. Cell cycle checkpoints, genomic integrity, and cancer. Cold Spring Harb Symp Quant Biol 1994;59:259–63.
[20] Tarry-Adkins JL, Ozanne SE. Mechanisms of early life programming: current knowledge and future directions. Am J Clin Nutr 2011.
[21] Bayarsaihan D. Epigenetic mechanisms in inflammation. J Dent Res 2011;90(1): 9–17.
[22] Woroniecki R, Gaikwad AB, Susztak K. Fetal environment, epigenetics, and pediatric renal disease. Pediatr Nephrol 2011;26(5):705–11. PMCID: 3063864.
[23] Licciardi PV, Wong SS, Tang ML, Karagiannis TC. Epigenome targeting by probiotic metabolites. Gut Pathog 2010;2(1):24. PMCID: 3016256.
[24] Gao Z, Xu CW. Glucose metabolism induces mono-ubiquitination of histone H2B in mammalian cells. Biochem Biophys Res Commun 2011;404(1):428–33.
[25] Fanelli M, Amatori S, Barozzi I, Soncini M, Dal Zuffo R, Bucci G, et al. Pathology tissue-chromatin immunoprecipitation, coupled with high-throughput sequencing, allows the epigenetic profiling of patient samples. Proc Natl Acad Sci USA 2010;107(50):21535–40. PMCID: 3003125.
[26] Berdasco M, Esteller M. Aberrant epigenetic landscape in cancer: how cellular identity goes awry. Dev Cell 2010;19(5):698–711.
[27] Larson JL, Yuan GC. Epigenetic domains found in mouse embryonic stem cells via a hidden Markov model. BMC Bioinformatics 2010;11:557. PMCID: 2992069.
[28] Burdge GC, Lillycrop KA. Bridging the gap between epigenetics research and nutritional public health interventions. Genome Med 2010;2(11):80. PMCID: 3016622.
[29] Marteau JB, Rigaud O, Brugat T, Gault N, Vallat L, Kruhoffer M, et al. Concomitant heterochromatinisation and down-regulation of gene expression unveils epigenetic silencing of RELB in an aggressive subset of chronic lymphocytic leukemia in males. BMC Med Genomics 2010;3:53. PMCID: 2994775.
[30] Xu XF, Du LZ. Epigenetics in neonatal diseases. Chin Med J (Engl) 2010;123(20): 2948–54.
[31] Lee J, Ryu H. Epigenetic modification is linked to Alzheimer's disease: is it a maker or a marker? BMB Rep 2010;43(10):649–55.
[32] McGee SL, Hargreaves M. Histone modifications and exercise adaptations. J Appl Physiol 2011;110(1):258–63.
[33] Atanassov BS, Koutelou E, Dent SY. The role of deubiquitinating enzymes in chromatin regulation. FEBS Lett 2010. PMCID: 3036772.
[34] Rumbaugh G, Miller CA. Epigenetic changes in the brain: measuring global histone modifications. Methods Mol Biol 2011;670:263–74.
[35] Rivera RM, Bennett LB. Epigenetics in humans: an overview. Curr Opin Endocrinol Diabetes Obes 2010;17(6):493–9.
[36] Osorio FG, Varela I, Lara E, Puente XS, Espada J, Santoro R, et al. Nuclear envelope alterations generate an aging-like epigenetic pattern in mice deficient in Zmpste24 metalloprotease. Aging Cell 2010;9(6):947–57.
[37] Taby R, Issa JP. Cancer epigenetics. CA Cancer J Clin 2010;60(6):376–92.
[38] Feinberg AP. Epigenetics at the epicenter of modern medicine. JAMA 2008;299(11): 1345–50.
[39] Yuasa Y. Frontiers in epigenetics medicine: foreword: miniseries assembled from a symposium organized by the Study Group on Epigenetics Medicine held at the 77th Annual Meeting of the Japanese Society for Hygiene, 2007. Environ Health Prev Med 2008;13(1):2. PMCID: 2698242.
[40] Iraola-Guzman S, Estivill X, Rabionet R. DNA methylation in neurodegenerative disorders: a missing link between genome and environment? Clin Genet 2011.
[41] Salbaum JM, Kappen C. Diabetic embryopathy: a role for the epigenome? Birth Defects Res A Clin Mol Teratol 2011.
[42] Sundar IK, Mullapudi N, Yao H, Spivack SD, Rahman I. Lung cancer and its association with chronic obstructive pulmonary disease: update on nexus of epigenetics. Curr Opin Pulm Med 2011.
[43] North ML, Ellis AK. The role of epigenetics in the developmental origins of allergic disease. Ann Allergy Asthma Immunol 2011;106(5):355–61.
[44] Jardim MJ. microRNAs: implications for air pollution research. Mutat Res 2011.
[45] Luco RF, Misteli T. More than a splicing code: integrating the role of RNA, chromatin and non-coding RNA in alternative splicing regulation. Curr Opin Genet Dev 2011.
[46] van Bokhoven H, Kramer JM. Disruption of the epigenetic code: an emerging mechanism in mental retardation. Neurobiol Dis 2010;39(1):3–12.
[47] Goldberg AD, Banaszynski LA, Noh KM, Lewis PW, Elsaesser SJ, Stadler S, et al. Distinct factors control histone variant H3.3 localization at specific genomic regions. Cell 2010;140(5):678–91. PMCID: 2885838.
[48] Scaffidi P, Misteli T. Cancer epigenetics: from disruption of differentiation programs to the emergence of cancer stem cells. Cold Spring Harb Symp Quant Biol 2010.
[49] Balkrishna SJ, Bhakuni BS, Chopra D, Kumar S. Cu-catalyzed efficient synthetic methodology for Ebselen and related Se–N heterocycles. Org Lett 2010;12(23): 5394–7.
[50] Oshita M, Takei Y, Kawano S, Fusamoto H, Kamada T. Protective effect of Ebselen on constrictive hepatic vasculature: prevention of alcohol-induced effects on portal pressure in perfused livers. J Pharmacol Exp Ther 1994;271(1):20–4.
[51] Schewe T. Molecular actions of Ebselen—an antiinflammatory antioxidant. Gen Pharmacol 1995;26(6):1153–69.
[52] Bhabak KP, Mugesh G. Synthesis, characterization, and antioxidant activity of some Ebselen analogues. Chemistry 2007;13(16):4594–601.
[53] Vempati RK. DNA damage in the presence of chemical genotoxic agents induce acetylation of H3K56 and H4K16 but not H3K9 in mammalian cells. Mol Biol Rep 2011.
[54] Schneider J, Bajwa P, Johnson FC, Bhaumik SR, Shilatifard A. Rtt109 is required for proper H3K56 acetylation: a chromatin mark associated with the elongating RNA polymerase II. J Biol Chem 2006;281(49):37270–4.
[55] Elledge SJ, Davis RW. DNA damage induction of ribonucleotide reductase. Mol Cell Biol 1989;9(11):4932–40. PMCID: 363644.
[56] Zhao X, Rothstein R. The Dun1 checkpoint kinase phosphorylates and regulates the ribonucleotide reductase inhibitor Sml1. Proc Natl Acad Sci USA 2002;99(6):3746–51. PMCID: 122595.
[57] Chabes A, Domkin V, Thelander L. Yeast Sml1, a protein inhibitor of ribonucleotide reductase. J Biol Chem 1999;274(51):36679–83.
[58] Zhao X, Chabes A, Domkin V, Thelander L, Rothstein R. The ribonucleotide reductase inhibitor Sml1 is a new target of the Mec1/Rad53 kinase cascade during growth and in response to DNA damage. EMBO J 2001;20(13):3544–53. PMCID: 125510.
[59] Andreson BL, Gupta A, Georgieva BP, Rothstein R. The ribonucleotide reductase inhibitor Sml1, is sequentially phosphorylated, ubiquitylated and degraded in response to DNA damage. Nucleic Acids Res 2010;38(19):6490–501. PMCID: 2965251.