REPAIR OF OXIDATIVE DAMAGE IN DNA AFTER PHYSICAL EXERCISE

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Abstract:

The author discusses the issues of widespread oxidative stress. The focus was mainly on DNA damage and their consequences. DNA repair mechanisms were also discussed, especially direct and indirect (BER, NER, NHEJ and HR) ones. An impact of physical effort on DNA damage was also presented. Finally, the evidence was provided that regular physical exercise initiates adaptation mechanisms and may participate in minimizing the negative effects of oxidative stress occurring naturally in cells.

INTRODUCTION

OXIDATIVE STRESS AND ROS

Stress is a condition that accompanies organisms at various stages of their growth. This term was introduced in the middle of the last century and means the state of disturbed homeostasis of the organism [Drabik, Stankiewicz 2006]. Factors that cause stress most often come from the external environment. Stimulating the body with a stressful factor affects on a series of defensive reactions aimed at limiting contact with the stressor. Their goal is also to restore the previous balance. One of types of stress affect on cells is oxidative stress (OS). OS is an imbalance between production and neutralization of reactive oxygen species (ROS). These particles have an oxygen atom in their structure and are easy to enter into chemical reactions. The formation of ROS can be induced by factors such as: UV rays, toxic compounds taken with food, alcohol consumption, cigarettes and even physical exertion [Lambrinoudaki et al. 2012].

Reactive oxygen species can affect the body in two ways. They can take part in signalling, induce of apoptosis or act bactericidal. They also participate in stimulating the work of the next elements of the immune system. This phenomenon is most often observed during various types of infections [Mittler 2017]. In addition to the positive effects on cells, ROS also induce a number of undesirable reactions. As the highly reactivated particles, reactive oxygen species may oxidize cell components which are necessary for cell proper functioning. Enzyme damage contributes to metabolic disturbances. By oxidizing proteins, ROS also affect transport across membranes. Oxidation of -SH groups in proteins results in change in their conformation and function, which also induces a number of undesirable effects in cells [Zhang et al. 2013]. Reactive oxygen species also easily react with lipids. In this case, main and side chains undergo peroxidation. In addition, ROS can induce breakage of lipids, which leads to the formation of further products harmful to cells. All these modifications affect the permeability of cell membranes which mainly components are fats. What is more, influencing the fluidity of the lipid bilayer results in a dramatic disruption of its

integrity [Barrera 2012]. The interaction of ROS with DNA leads to the formation of oxidative modification of nitrogen bases. This compounds have altered stability and can take part in further reaction causing another damage. The presence of an oxidized nucleotide in the chain may also induce defect during replication. For example, 8-hydroxy-2-deoxyguanosine (8-OHdG) is an oxidative modification of guanosine that forms stable binding to adenosine (A). After two rounds of replication there will be a transversion involving the incorporation of thymine instead of guanine (G → T) [Zaremba, Olinski 2010]. In addition, ROS may induce single or double strand breaks in the DNA strand. Under limited oxygen concentration, a hydrogen atom is detached from the C4 carbon. This reaction causes breaking the phosphodiester binding in the molecules and leads to DNA breakage within one of the strands (SSB). Under oxygen conditions, the consequences of ROS on DNA are more toxic to cells. In this case, not only the hydrogen atoms at C4, but also the remaining atoms can be detached. Reactions leading to breaks of bonds at other sites in the molecule cause the opening of the deoxyribose ring which affect into the occurrence of double-strand breaks in DNA molecules (DBS) [Cadet, Wager 2013].

MECHANISMS OF DNA REPAIR

ROS can induce nucleic acid damage, as it was mentioned. Oxidative defects of this type are very dangerous for cells. Damage to DNA causes changes in the proper functioning of cells affect on functional disorders, divisions or the induction of mutagenesis. Damage within the DNA is often so severe that cell death is initiated instead of defect repair [Lodish et al. 2008].

Due to the prevalence of oxidative stress, organisms have developed mechanisms that minimize the negative effects of OS. Depending on the damage that occurs, the cells can induce different DNA repair mechanisms, which can generally be divided into: direct and indirect. The first group are damage repair systems in which the continuity of the sugar-phosphate chain was not interrupted. For example, ligase is a type of enzyme which facilitates the joining of DNA ends together. By using ATP, it is possible to restore phosphodiester bonds in double-stranded DNA [Sancar et al. 2004]. Another mechanism that should be mentioned is the removal of alkyl groups from the bases of DNA. With the use of methyltranferases, the alkyl groups are transferred to the MGMT protein (O6-methyl-guanine DNA methyltransferase), which degrades after such irreversible modification. The mechanism requires continuous synthesis of MGMT protein, which makes it very expensive for the cell [Srivenugopal, Ali-Osman 2002; Jena 2012].

Mechanisms for indirect repair of single DNA breaks include 2 main pathways: BER (*base excision repair*) and NER (*nucleotide excision repair*). Repair, using these pathways, takes place in the cell without disturbing physiological processes. The DSB repair mechanisms include: NHEJ (*nonhomologous end-joining*) and HR (*homologous recombination repair*). As mentioned earlier, DNA damage of this type is highly toxic to cells and is often associated with stopping in a given phase of the cell cycle.

BER MECHANISM

Literature data show that the most commonly used mechanism for repairing oxidative damage is the BER pathway. This mechanism is based on cutting the base, thereby correcting minor changes in the structure of DNA. These can be oxidized bases, deamination, alkylation or apurinic/apyrimidinic sites (AP) [Gomes et al. 2017]. BER can run through 2 mechanisms.

The first of them, also called as the "GO" system, uses hMTH1 phosphohydrolase. This enzyme degrades 8-OHdG from the pool of nucleotides available in the cell. It prevents in this way the improper incorporation of oxidative modification of the nitrogenous base into the newly created DNA strand [Zaremba, Oliński 2010].

The second possible pathway is the main BER repair mechanism. It runs in the cell nucleus and mitochondria. It is believed that this is possible due to the presence of different isoforms or genetically distant proteins. Literature data show that depending on the DNA modification occurring, the stage of recognizing a modified nitrogenous base is initiated by one of at least 11 enzymes called glycosylases [Krokan, Bjoras 2013]. After identifying the damage, the inappropriate nucleotide or its oxidised form is removed. Removal of damage nucleotide form DNA using highly conserved glycosylation enzymes. For example, OGG1 (8-oxoguanine glycosylase) is a protein which mediates the removal of 8-OHdG from the sugar skeleton without inducing breaks in the DNA strand. After base removal, AP site is created. Next, to apurinic/apyrimidinic site, endonucleases AP are attached. The most commonly known in these group of enzymes is APE1. This endonuclease not only participates in the BER repair pathway but is additionally a factor regulating gene expression. APE1 initiates the 3'OH free end formation by breaking the phosphodiester bond towards to 5 ' end from the AP site [Tell et al. 2009]. Next, the deoxyribose molecule is removed from the chain as a result of the activity of the β polymerase (Pol β). Depending on the size of the missing fragment, 2 different paths are initiated. In the absence of a single nucleotide, the defects are repaired by Pol β. Ligase III mediates the joining of free DNA ends. When larger strands occur in the strand, another repair path is activated. The 5 ' end is moved away. To add a missing fragment, replication proteins are necessary (PCNA/RFC, Pol δ lub Polε). After completion of the synthesis, the free 5’ end is degraded by the FEN1 endonuclease, and the complementary strands are joined using the Ligase I activity [Lindhal, 2000].

NER MECHANISM

NER is a mechanism used mainly to repair larger parts of DNA stand. Its activation also takes place in response to the changed in DNA structure. They may be thymidine dimers as well as single strand breaks. Literature data indicate that NER corrects lengths around 30 nucleotides. NER is a BER support system when glycosylases are damaged. What is more, NER compared with BER does not occurs in the mitochondria. Within the NER pathway it can be distinguish two subordinate mechanisms: TC-NER and GG-NER. These mechanisms are activated in response to different factors. Their initial stages are different, but the final stages proceed in the same way [Marteijn et al. 2014].

TC-NER is mainly related to the repair of damage detected during gene transcription. This mechanism is fast and its activation occurs in response to stopping of RNA polymerase II at the site of the thread defect. Interruption of the polymerase's work is a signal initiating the organization of protein complexes. The first activated proteins are CSA and CSB. CSB is a protein that has the ability to bind to DNA. What is more, it interacts with Pol RNA II by moving it and facilitating access to other damage to the site of damage [Citterio et al. 2000; Lake, Fan 2013]. CSA is a protein that has the ability to interact with other proteins. In the case of the TC-NER pathway, CSA joins Pol II RNA, and due to ubiquitin ligase activity, it degrades the enzyme [Groisman et al. 2003; Fousteri et al. 2006; Chitale, Richly 2017]. CSA and CSB participate in the activation and recruitment of subsequent repairing factors. Interestingly is that the CSA with the DDB1, CUL4, and RBX1 proteins are part of the $CRL4^{CSA}$ complex. This complex together with the CSB protein participate in attracting

damage and activating subsequent proteins necessary for DNA repair. After the construction of the CRL4 ^{CSA} complex, the further steps of TC-NER are the same as for GG-NER and will be discussed later [Saijo 2013; Melis et al. 2011].

GG-NER also called the global genome repair pathway, is a long-term process and mainly affects on transcriptionally inactive sequences. Literature data indicate that the genome is constantly searched for changes in the DNA helix through the XPC and DDB2 proteins. The first of them recognizes specific, distorted structures within the strand. After finding the defect, XPC changes its conformation increasing its ability to binding for DNA and subsequent proteins of the NER system. XPC can also form a complex with HR23B and Centrin2 [Melis et al. 2011]. The presence of other factors provides complex stability and further increases the affinity for DNA. Literature data indicate that Centrin2 is a protein necessary for the proper NER mechanism [Nishi et al. 2005]. The DDB2 protein mainly recognizes DNA pyrimidine dimers and acts homologous to the CSA participating in TC-NER. DDB2 interacts with the same factors creating a complex called CRL4 ^{DDB2} consisting of: DDB2-DDB1-CUL4-RBX1 [Feltes et al. 2018].

CRL4 ^{CSA} for TC-NER and CRL4 ^{DDB2} from the GG-NER pathway have the ability to interact with the TFIIH factor. It is a complex of 10 proteins, which consists of: XPB, XPD, p62, p52, p44, p34, p8, MAT1, CDK7 and cyclin H [Compe, Egly 2012]. The main task of TFIIH is to create the structure also called transcription bubble at the site of DNA damage. A special role is played by XPB and XPD. These molecules have helicase activity, opening the double structure of the DNA strand near the defect [Fuss, Tainer 2011]. XPA, RPA and XPG participating in the organization of the repair initiation complex, join in the area of damage, which significantly increases the positioning of repair proteins around the changes. Literature data suggest that XPA is a kind of platform for joining next proteins and together with RPA performs the protective function of an undamaged strand [Fadda 2016; Sugitani et al. 2014]. Additionally, XPA interacts with the XPG and ERCC1-XPF edonucleases which activity is necessary for the double incision of the damaged DNA strand. XPG initiates cutting strand at the 3 'end, and ERCC1-XPF at the 5' end. As a result of action of these proteins in the NER pathway, a damaged sequence lengths around of 24-32 nucleotides is removed [Scharer 2008; Spivak 2015].

NHEJ MECHANISM

As mentioned earlier, in addition to SSB as a result of harmful factors, double-stranded DNA strands may also be induced. NHEJ (nonhomologous end-joining) is the most commonly used mechanism among others to repair double cracks in DNA. This pathway does not require the presence of homologous sequences to repair damage. NHEJ initiation is observed at various stages of the cell cycle [Davis, Chen 2013].

The mechanism starts with finding a DSB site in the DNA strand. This is possible due to the presence of KU70 (XRCC6) and KU80 (XRCC5) proteins in the cells that form a heterodimer. The KU70 protein has the ability to bind to DNA through the occurrence of a specific SAP domain in its structure [Zhang et al. 2001; Fell, Schild-Poulter 2012]. KU80 interacts with subsequent proteins to form specific protein-protein bonds. Heterodimer KU70 / 80 binds to the free ends of the strand, keeping them close and additionally protects them against degradation [Zhang et al. 2004]. The next stage of the NHEJ pathway is the formation of the DNA-PK complex. It includes the KU70 / 80 dimer and the catalytic subunit from serine-threonine kinase DNA-PKcs. Attaching the DNA-PKcs subunit to the heterodimer induces a KU70 / 80 shift of about 10 base pairs, to facilitate access to damage to further

repair agents [Yoo, Dynan 1999]. After the formation of the DNA-PK complex, it becomes an active form of kinase [Dobbs et al. 2010]. Among its substrates many proteins can be mentioned. However, the most important for damage repair are: XRCC4, KU70, KU80 and DNA-PKCs [Calsou et al. 2003; Don et al. 2017].

Another protein that should be mentioned is Artemis. This protein belong to the family of metal-β-lactamases and has exonuclease activity 5 '→ 3'. Phosphorylation by the DNA-PKCs kinase of the Artemis protein results in the enzyme exhibiting 5 '→ 3' endonuclease properties manifested by the ability to cut double-stranded hairpin structure [Poplawski et al. 2009; Chang, Lieber 2016]. The enzonuclease activity consists in removing single-stranded fragments from each end. As its result, short deletions may be generated at the point of the thread break. The consequence of Artemis activity is the preparation of both DNA strands for ligation. The DNA polymerases μ and λ complement the missing nucleotides [Capp et al. 2006]. An important role in NHEJ is also played by bifunctional phosphatase / polynucleotide kinase (PNKP). It works by removing phosphate groups from the 3 'ends and by phosphorylation of the groups at the 5' end of the DNA strand. What is more, PNKP has the ability to interact with the XRCC4 protein, which is necessary for the proper DNA ligation [Dumitrache, McKinnon 2017]. XRCC4 together with ligase IV form a complex that is additionally aided by XLF (Cernunnos). Literature data indicate that Cernunnos is a protein necessary to stimulate the combination of non-complementary or partially complementary free blunt of the strands [Tsai et al. 2007]. Interestingly is that the XLF and XRCC4 are involved in the final stage of the NHEJ mechanism. By stimulating the activity of Ligase IV, they participate in the proper ligation of free ends [Gu et al. 2007; Davis, Chen 2013].

HR MECHANISM

HR is the repair of double-stranded DNA damage by searching for homology. The process is longer than the NHEJ repair path. Literature data show that the HR mechanism is more accurate and does not generate deletions at the site of damage [Sonoda et al. 2006]. The HR mechanism is initiated mainly during the S and G2 phases of the cell cycle. In this phases the sister chromatids are present. This structure are often unmodified complementary strand of the DNA which can be useful as a template on which the missing fragment is being added [Davis, Chen 2013].

After finding a DSB within the DNA strand, a process called end resection is started. It consists in creating single-stranded 3 'ends through nucleolytic degradation of the strand in the 5' → 3'. The main role in modifying the ends of DNA is attributed to the MRN complex, which consists of the following proteins: MRE11, RAD50 and NBS1 [Lamarche et al. 2010; Mimitou and Symington, 2011]. MRE11 is a nuclease generating single-stranded 3 'ends. Interestingly, its activity can be increased by the CTIP protein. Literature data suggest that the protein not only accelerates HR repair, but is also required for the proper HR process [Sartori et al. 2007]. The role of the RAD50 protein is to keep the ends of the DNA strand in the correct position. The ssDNA resection obtained after the end is a kind of platform for the joining of repair proteins.

The next step of HR is to attach to the free 3 'ends of the RPA protein (replication protein A). Its task is to prevent the connection of a developed thread or the formation of secondary structures [Zuo, Elledg 2003]. The RAD52 protein also participates in the HR mechanism. Its role is to counteract nuclease enzymes attacks and to supply RAD51 protein to the DNA strand [Grimme et al. 2010]. An important role is also played by BRC2 proteins, which promotes the attachment of RAD51 to ssDNA and the formation of so-called

nucleofilaments [Holloman 2011]. Nucleofilaments participate in the search for a homologous fragment and facilitate the attachment of subsequent repairing factors. These can be: RAD54, RAD51B, RAD51C, RAD51D, XRCC2 and XRCC3 [Brenneman et al. 2000; Mazin et al. 2010]. These proteins form complexes that not only participate in the exchange of homologous strands, but also catalyze the joining of single-stranded fragments.

RAD51 also participates in creating nucleophilaments, initiates the creation of so-called D loop (*displacement loop*) necessary for correct HR repair. This type of structure facilitates the action of DNA polymerases that extend the 3 'end. As a template for their activity may be used sister chromatid. After adding the missing fragment, the repaired DNA strand is released by helicases enzymes. The final stage of the HR mechanism is the ligation of 3 'and 5' free ends with phosphodiester-binding by using ligase enzymes[Li, Heyer 2008; Heyer et al. 2010].

OXIDATIVE STRESS VS PHYSICAL EXERCISE

Physical effort is associated with an increased demand for energy, which results in ROS overproduction. Occurrence of oxidative stress in skeletal muscles during exercise is therefore a common phenomenon, depending on many factors. The production of ROS in these tissues is associated primarily with the functioning of mitochondria, NADPH oxidase and xanthine oxidase. An additional source of ROS may be elements of the immune system, mainly macrophages and neutrophils. These cells are activated during exercise in response to muscular micro-injuries. Oxidative burst, or ROS overproduction carried out by elements of the immune system is one of the basic defence mechanisms of the body [Steinbacher, Eckl 2015]. This type of response to physical effort is long-lasting. It aims at remove damaged cellular components and restore balance from oxidative stress [Tidball 2005].

The increase in the level of reactive oxygen species during physical exercise can have a dual function. By oxidizing biomolecules, ROS contribute to the damage and degradation of cellular components, which can significantly reduce the physical capacity of the body [Powers, Jackson 2008; 2011]. A high level of reactive oxygen species is also observed in many diseases, such as lung diseases, cancer, atherosclerosis etc. Literature data indicate that the negative effects of ROS on the body occur mainly during very intense physical exertion. When the training is appropriately suited to the body's endurance capacity, the positive effect of reactive oxygen species is observed [Moore et al. 2012]. The positive result is the remodelling of skeletal muscles. As already mentioned, ROS activates the immune system to remove defective cellular components, which significantly affects the reorganization of muscle tissue. What is more, regular exercises mediate the adaptation of antioxidant systems and repair systems to the regularly high ROS level occurring. The consequence of regular physical exercise may be reduced number of oxidative damage and increased resistance to oxidative stress [Radak et al. 2001, 2005; Florida-James et al. 2016].

Literature data also indicate that not only the intensity, but also the type of training and the age of the athlete affect the level of ROS. It has been observed that amongst people in their fifties leading to a sedentary lifestyle so far, the OS generated during physical exercise contributes to the accumulation of oxidative damage. But for people who do sport, exercise may be a helpful strategy to attenuate the age-related decline [Gonzalo-Calco et al. 2013]. Studies have also been carried out to compare the effect of high altitudes training on oxidative stress levels. It has been observed that high altitudes training contributes to increased DNA damage and lipid peroxidation compared to physical activity in normal conditions [Moller et al. 2001; Bakonyi, Radak 2004]. From the examples presented above, it can be concluded that

ROS production and the occurrence of oxidative stress are necessary in order to achieve adaptation to higher exercise loads.

INCREASE OF OXYDATE DNA DAMAGE LEVELS DURING EXERCISE

Literature data show that not only the ROS level increases during exercise, but also the concentration of compounds serving as markers of oxidative stress. One of them is previously mentioned 8-OHdG. It has been shown that amongst people who have trained the retina for at least 3 years, the concentration of 8-OHdG in athletes' serum increases after exercise. An increase in the level of this marker clearly indicates an increase in DNA damage after physical exercise. Interestingly, it was not observed that the high level of 8-OHdG influenced significantly the sports results of the subjects. There were no significant changes in the total antioxidant capacity of the body. It is assumed that this is related to the level of training of people participating in the experiment [Radojewski et al. 2018].

A similar group of researchers has demonstrated a similar phenomenon in their work. They checked the amount of DNA damage and the ability to repair them. The researchers were working on a group of Washington residents at 50-76 age. Intensity of exercises was selected, based on the BMI parameter. The study showed that moderate training (150 minutes per week) does not cause a significant increase in the number of DNA damage. However, the researchers observed a different, interesting phenomenon. It was tried to compare DNA repair after 15- and 60-minutes from the occurrence of a harmful factor. It has been proven that well-chosen, regular training leads to an increase in the repair of DNA damage resulting from oxidative stress [Cash et al. 2014]. The results discussed are consistent with those presented by Soares et al. The researchers observed that people exercising regularly after physical exertion have less DNA damage compared to sedentary people. What is more, a decrease in the level of other markers of oxidative stress (e.g. lipid peroxidation) has also been demonstrated [Soares et al., 2015]. These results may support the thesis that regular physical exercise initiates adaptation mechanisms and may participate in minimizing the negative effects of OS occurring naturally in cells.

How important is physical effort among people suffering from various diseases, have demonstrated da Silva et al. The research was carried out on people with COPD (chronic obstructive pulmonary disease). The researchers observed a similar trend as the aforementioned groups. They proved that in patients who regularly exercise sports there is a significant increase in the repair of DNA damage. What is more, after physical exertion a lower concentration of 8-OHdG was demonstrated among people training compared to the group of people with COPD who do not practice sport [da Silva et al., 2013].

Sport seems to have beneficial systemic of 'anti-aging' effects. Such conclusions were suggested on the basis of many studies [Mergener et al., 2009; Werner et al., 2009]. Dimauro et al. demonstrated that 12-week physical training affects the decline in the level of cell-aging markers and proteins involved in cell death processes [Dimauro et al. 2016]. Another group of researchers also confirmed this thesis. It has been proved that after exercise, the levels of KU70 and KU80 proteins involved in the NHEJ repair mechanism increase. Their results may indicate that after oxidative stress among athletes, oxidative damage repair pathways in cells are preferred than initiation of cell apoptosis [Laye et al. 2012].

BIBLIOGRAPHY:

1. Bakonyi T., Radak Z. (2004): *High altitude and free radicals.* In Journal of sports science & medicine 3 (2), pp. 64–69.
2. Barrera G. (2012): *Oxidative stress and lipid peroxidation products in cancer progression and therapy.* In ISRN oncology 2012, p. 137289. DOI: 10.5402/2012/137289.
3. Brenneman M. A., Weiss A. E., Nickoloff J. A., Chen D. J. (2000): *XRCC3 is required for efficient repair of chromosome breaks by homologous recombination.* In Mutation research 459 (2), pp. 89–97.
4. Cadet J., Wagner J. R. (2013): *DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation.* In Cold Spring Harbor perspectives in biology 5 (2). DOI: 10.1101/cshperspect.a012559.
5. Calsou P., Delteil C., Frit P., Drouet J., Salles B. (2003): *Coordinated assembly of Ku and p460 subunits of the DNA-dependent protein kinase on DNA ends is necessary for XRCC4-ligase IV recruitment.* In Journal of molecular biology 326 (1), pp. 93–103.
6. Capp J. P., Boudsocq F., Bertrand P., Laroche-Clary A., Pourquier P., Lopez B. S. et al. (2006): *The DNA polymerase lambda is required for the repair of non-compatible DNA double strand breaks by NHEJ in mammalian cells.* In Nucleic acids research 34 (10), pp. 2998–3007. DOI: 10.1093/nar/gkl380.
7. Cash S. W., Beresford S. A., Vaughan T. L., Heagerty P. J. Bernstein L., White E., Neuhouser M. L. (2014): *Recent physical activity in relation to DNA damage and repair using the comet assay.* In Journal of physical activity & health 11 (4), pp. 770–776. DOI: 10.1123/jpah.2012-0278.
8. Chang H. H. Y., Lieber M. R. (2016): *Structure-Specific nuclease activities of Artemis and the Artemis. DNA-PKcs complex.* In Nucleic acids research 44 (11), pp. 4991–4997. DOI: 10.1093/nar/gkw456.
9. Chitale S., Richly H. (2017): *Timing of DNA lesion recognition. Ubiquitin signaling in the NER pathway.* In Cell cycle (Georgetown, Tex.) 16 (2), pp. 163–171. DOI: 10.1080/15384101.2016.1261227.
10. Citterio E., van den Boom V., Schnitzler G., Kanaar R., Bonte E., Kingston R. E. et al. (2000): *ATP-dependent chromatin remodeling by the Cockayne syndrome B DNA repair-transcription-coupling factor.* In Molecular and cellular biology 20 (20), pp. 7643–7653.
11. Compe E., Egly J. M. (2012): *TFIIH. When transcription met DNA repair.* In Nature reviews. Molecular cell biology 13 (6), pp. 343–354. DOI: 10.1038/nrm3350.
12. da Silva A. L. G., da Rosa H. T., Bender E., da Rosa P. R., Salvador M., Charlier C. F. et al. (2013): *Effect of Physical Exercise on the Level of DNA Damage in Chronic Obstructive Pulmonary Disease Patients.* In ISRN Pulmonology 2013 (2 i), pp. 1–8. DOI: 10.1155/2013/907520.
13. Davis A. J., Chen D. J. (2013): *DNA double strand break repair via non-homologous end-joining.* In Translational cancer research 2 (3), pp. 130–143. DOI: 10.3978/j.issn.2218-676X.2013.04.02.
14. Dimauro I., Scalabrin M., Fantini C., Grazioli E., BeltranV., Maria R., Mercatelli N. et al. (2016): *Resistance training and redox homeostasis. Correlation with age-*

associated genomic changes. In Redox biology 10, pp. 34–44. DOI: 10.1016/j.redox.2016.09.008.

15. Dobbs T. A., Tainer J. A., Lees-Miller S. P. (2010): *A structural model for regulation of NHEJ by DNA-PKcs autophosphorylation.* In DNA repair 9 (12), pp. 1307–1314. DOI: 10.1016/j.dnarep.2010.09.019.
16. Dong J., Zhang T., Ren Y., Wang Z., Ling C. C., He F. et al. (2017): *Inhibiting DNA-PKcs in a non-homologous end-joining pathway in response to DNA double-strand breaks.* In Oncotarget 8 (14), pp. 22662–22673. DOI: 10.18632/oncotarget.15153.
17. Drabik L., Stankiewicz A. (2006): *Słownik języka polskiego PWN.* Warszawa: Wydawnictwo Naukowe PWN; Wydawnictwo Naukowe PWN.
18. Dumitrache L. C., McKinnon P. J. (2017): *Polynucleotide kinase-phosphatase (PNKP) mutations and neurologic disease.* In Mechanisms of ageing and development 161 (Pt A), pp. 121–129. DOI: 10.1016/j.mad.2016.04.009.
19. Fadda E. (2016): *Role of the XPA protein in the NER pathway. A perspective on the function of structural disorder in macromolecular assembly.* In Computational and structural biotechnology journal 14, pp. 78–85. DOI: 10.1016/j.csbj.2015.11.007.
20. Fell V. L., Schild-Poulter C. (2012): *Ku regulates signaling to DNA damage response pathways through the Ku70 von Willebrand A domain.* In Molecular and cellular biology 32 (1), pp. 76–87. DOI: 10.1128/MCB.05661-11.
21. Feltes B. C., Pedebos C., Bonatto D., Verli H. (2018): *Dynamics of DDB2-DDB1 complex under different naturally-occurring mutants in Xeroderma Pigmentosum disease.* In Biochimica et biophysica acta. General subjects 1862 (12), pp. 2579–2589. DOI: 10.1016/j.bbagen.2018.08.007.
22. Florida-James G. D., Simpson R., Davison G., Close G. (2016): *Exercise, Free Radical Metabolism, and Aging. Cellular and Molecular Processes.* In Oxidative medicine and cellular longevity 2016, p. 3813680. DOI: 10.1155/2016/3813680.
23. Fousteri M., Mullenders L. H. F. (2008): *Transcription-coupled nucleotide excision repair in mammalian cells. Molecular mechanisms and biological effects.* In Cell research 18 (1), pp. 73–84. DOI: 10.1038/cr.2008.6.
24. Fousteri M.,; Vermeulen W., van Zeeland A. A., Mullenders L. H. F. (2006): *Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo.* In Molecular cell 23 (4), pp. 471–482. DOI: 10.1016/j.molcel.2006.06.029.
25. Fuss J. O., Tainer J. A. (2011): *XPB and XPD helicases in TFIIH orchestrate DNA duplex opening and damage verification to coordinate repair with transcription and cell cycle via CAK kinase.* In DNA repair 10 (7), pp. 697–713. DOI: 10.1016/j.dnarep.2011.04.028.
26. Gomes L. R., Menck Carlos F. M., Leandro G. S. (2017): *Autophagy Roles in the Modulation of DNA Repair Pathways.* In International journal of molecular sciences 18 (11). DOI: 10.3390/ijms18112351.
27. Gonzalo-Calvo D., Fernández-García B., Luxán-Delgado B., Rodríguez-González S., García-Macia M., Suárez F. M. et al. (2013): *Chronic training increases blood oxidative damage but promotes health in elderly men.* In Age (Dordrecht, Netherlands) 35 (2), pp. 407–417. DOI: 10.1007/s11357-011-9358-6.

28. Grimme J. M., Honda M., Wright R., Okuno Y., Rothenberg E., Mazin A. V. et al. (2010): *Human Rad52 binds and wraps single-stranded DNA and mediates annealing via two hRad52-ssDNA complexes.* In Nucleic acids research 38 (9), pp. 2917–2930. DOI: 10.1093/nar/gkp1249.

29. Groisman R., Polanowska J., Kuraoka I., Sawada J., Saijo M., Drapkin R. et al. (2003): *The Ubiquitin Ligase Activity in the DDB2 and CSA Complexes Is Differentially Regulated by the COP9 Signalosome in Response to DNA Damage.* In Cell 113 (3), pp. 357–367. DOI: 10.1016/S0092-8674(03)00316-7.

30. Gu J., Lu H., Tsai A. G., Schwarz K., Lieber M. R. (2007): *Single-stranded DNA ligation and XLF-stimulated incompatible DNA end ligation by the XRCC4-DNA ligase IV complex. Influence of terminal DNA sequence.* In Nucleic acids research 35 (17), pp. 5755–5762. DOI: 10.1093/nar/gkm579.

31. Heyer W. D., Ehmsen K. T., Liu J. (2010): *Regulation of homologous recombination in eukaryotes.* In Annual review of genetics 44, pp. 113–139. DOI: 10.1146/annurev-genet-051710-150955.

32. Holloman W. K. (2011): *Unraveling the mechanism of BRCA2 in homologous recombination.* In Nature structural & molecular biology 18 (7), pp. 748–754. DOI: 10.1038/nsmb.2096.

33. Jena N. R. (2012): *DNA damage by reactive species. Mechanisms, mutation and repair.* In J Biosci 37 (3), pp. 503–517. DOI: 10.1007/s12038-012-9218-2.

34. Krokan H. E., Bjørås M. (2013): *Base excision repair.* In Cold Spring Harbor perspectives in biology 5 (4), a012583. DOI: 10.1101/cshperspect.a012583.

35. Lake R. J., Fan H. Y. (2013): *Structure, function and regulation of CSB. A multi-talented gymnast.* In Mechanisms of ageing and development 134 (5-6), pp. 202–211. DOI: 10.1016/j.mad.2013.02.004.

36. Lamarche B. J., Orazio N. I., Weitzman M. D. (2010): The *MRN complex in double-strand break repair and telomere maintenance.* In FEBS letters 584 (17), pp. 3682–3695. DOI: 10.1016/j.febslet.2010.07.029.

37. Lambrinoudaki I., Ceasu I., Depypere H., Erel T., Rees M., Schenck-Gustafsson K. et al. (2013): *EMAS position statement. Diet and health in midlife and beyond.* In Maturitas 74 (1), pp. 99–104. DOI: 10.1016/j.maturitas.2012.10.019.

38. Laye M. J., Solomon T. P. J., Karstoft K., Pederse, K. K., Nielsen S. D., Pedersen B. K. (2012): *Increased shelterin mRNA expression in peripheral blood mononuclear cells and skeletal muscle following an ultra-long-distance running event.* In Journal of applied physiology (Bethesda, Md. : 1985) 112 (5), pp. 773–781. DOI: 10.1152/japplphysiol.00997.2011.

39. Li X., Heyer W. D. (2008): *Homologous recombination in DNA repair and DNA damage tolerance.* In Cell research 18 (1), pp. 99–113. DOI: 10.1038/cr.2008.1.

40. Lindahl T. (2000): *Suppression of spontaneous mutagenesis in human cells by DNA base excision-repair.* In Mutation research 462 (2-3), pp. 129–135.

41. Lodish H., Berk A.; Zipursky S. L., Matsudaira P., Baltimore D., Darnell J. (2008): *Molecular cell biology*. 6th ed., 2nd print. New York: W. H. Freeman and Co.

42. Marteijn J. A., Lans H., Vermeulen W., Hoeijmakers J. H. J. (2014): *Understanding nucleotide excision repair and its roles in cancer and ageing.* In Nature reviews. Molecular cell biology 15 (7), pp. 465–481. DOI: 10.1038/nrm3822.

43. Mazin A. V., Mazina O. M., Bugreev D. V., Rossi M. J. (2010): *Rad54, the motor of homologous recombination.* In DNA repair 9 (3), pp. 286–302. DOI: 10.1016/j.dnarep.2009.12.006.

44. Melis J. P. M., Luijten M., Mullenders L. H. F., van Steeg H. (2011): *The role of XPC. Implications in cancer and oxidative DNA damage.* In Mutation research 728 (3), pp. 107–117. DOI: 10.1016/j.mrrev.2011.07.001.

45. Mergener M., Martin, M. R., Antunes M. V., da Silva C. C., Lazzaretti C., Fontanive T. O. et al. *(2009): Oxidative stress and DNA damage in older adults that do exercises regularly.* In Clinical biochemistry 42 (16-17), pp. 1648–1653. DOI: 10.1016/j.clinbiochem.2009.08.001.

46. Mimitou E. P., Symington L. S. (2011): *DNA end resection--unraveling the tail.* In DNA repair 10 (3), pp. 344–348. DOI: 10.1016/j.dnarep.2010.12.004.

47. Mittler R. (2017): *ROS Are Good.* In Trends in plant science 22 (1), pp. 11–19. DOI: 10.1016/j.tplants.2016.08.002.

48. Møller P., Loft S., Lundby C., Olsen N. V. (2001*): Acute hypoxia and hypoxic exercise induce DNA strand breaks and oxidative DNA damage in humans.* In FASEB journal : official publication of the Federation of American Societies for Experimental Biology 15 (7), pp. 1181–1186.

49. Moore S. C., Patel A. V., Matthews C. E., Berrington de Gonzalez A., Park Y., Katki H. A. et al. (2012): *Leisure time physical activity of moderate to vigorous intensity and mortality. A large pooled cohort analysis.* In PLoS medicine 9 (11), e1001335. DOI: 10.1371/journal.pmed.1001335.

50. Nishi R., Okuda Y., Watanabe E., Mori T., Iwai S., Masutani C. et al. (2005): *Centrin 2 stimulates nucleotide excision repair by interacting with xeroderma pigmentosum group C protein.* In Molecular and cellular biology 25 (13), pp. 5664–5674. DOI: 10.1128/MCB.25.13.5664-5674.2005.

51. Powers S. K., Jackson M. J. (2008): *Exercise-induced oxidative stress. Cellular mechanisms and impact on muscle force production.* In Physiological reviews 88 (4), pp. 1243–1276. DOI: 10.1152/physrev.00031.2007.

52. Powers S. K., Talbert E. E., Adhihetty P. J. (2011): *Reactive oxygen and nitrogen species as intracellular signals in skeletal muscle.* In The Journal of physiology 589 (Pt 9), pp. 2129–2138. DOI: 10.1113/jphysiol.2010.201327.

53. Radak Z., Taylor A. W., Ohno H., Goto S. (2001): *Adaptation to exercise-induced oxidative stress. From muscle to brain.* In Exercise immunology review 7, pp. 90–107.

54. Radak Z., Chung Hae Y., Goto S. (2005): *Exercise and hormesis. Oxidative stress-related adaptation for successful aging.* In Biogerontology 6 (1), pp. 71–75. DOI: 10.1007/s10522-004-7386-7.

55. Radojewski M., Podgórski T., Pospieszna B., Kryściak J., Śliwicka E., Karolkiewicz J. (2018): *Skeletal Muscle Cell Damage Indicators in Volleyball Players after the Competitive Phase of the Annual Training Cycle.* In Journal of human kinetics 62, pp. 81–90. DOI: 10.1515/hukin-2017-0160.

56. Saijo M. (2013): *The role of Cockayne syndrome group A (CSA) protein in transcription-coupled nucleotide excision repair.* In Mechanisms of ageing and development 134 (5-6), pp. 196–201. DOI: 10.1016/j.mad.2013.03.008.

57. Sancar A., Lindsey-Boltz L. A., Unsal-Kaçmaz K., Linn S. (2004): *Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints.* In Annual review of biochemistry 73, pp. 39–85. DOI: 10.1146/annurev.biochem.73.011303.073723.

58. Sartori A. A., Lukas C., Coates J., Mistrik M., Fu S., Bartek J. et al. (2007): *Human CtIP promotes DNA end resection.* In Nature 450 (7169), pp. 509–514. DOI: 10.1038/nature06337.

59. Schärer O. D. (2008): *XPG. Its products and biological roles.* In Advances in experimental medicine and biology 637, pp. 83–92.

60. Schärer O. D. (2013): *Nucleotide excision repair in eukaryotes.* In Cold Spring Harbor perspectives in biology 5 (10), a012609. DOI: 10.1101/cshperspect.a012609.

61. Soares J. P., Silva A. M., Oliveira M. M., Peixoto F., Gaivão I., Mota M. P. (2015): *Effects of combined physical exercise training on DNA damage and repair capacity. Role of oxidative stress changes.* In Age (Dordrecht, Netherlands) 37 (3), p. 9799. DOI: 10.1007/s11357-015-9799-4.

62. Sonoda E., Hochegger H., Saberi A., Taniguchi Y., Takeda S. (2006): *Differential usage of non-homologous end-joining and homologous recombination in double strand break repair.* In DNA repair 5 (9-10), pp. 1021–1029. DOI: 10.1016/j.dnarep.2006.05.022.

63. Spivak G. (2015): *Nucleotide excision repair in humans.* In DNA repair 36, pp. 13–18. DOI: 10.1016/j.dnarep.2015.09.003.

64. Srivenugopal K. S., Ali-Osman F. (2002): *The DNA repair protein, O(6)-methylguanine-DNA methyltransferase is a proteolytic target for the E6 human papillomavirus oncoprotein.* In Oncogene 21 (38), pp. 5940–5945. DOI: 10.1038/sj.onc.1205762.

65. Steinbacher P., Eckl P. (2015): *Impact of oxidative stress on exercising skeletal muscle.* In Biomolecules 5 (2), pp. 356–377. DOI: 10.3390/biom5020356.

66. Sugitani N., Shell S. M., Soss S. E., Chazin W. J. (2014): *Redefining the DNA-binding domain of human XPA.* In Journal of the American Chemical Society 136 (31), pp. 10830–10833. DOI: 10.1021/ja503020f.

67. Tell G., Quadrifoglio F., Tiribelli C., Kelley M. R. (2009): *The many functions of APE1/Ref-1. Not only a DNA repair enzyme.* In Antioxidants & redox signaling 11 (3), pp. 601–620. DOI: 10.1089/ars.2008.2194.

68. Tidball J. G. (2005): *Inflammatory processes in muscle injury and repair.* In American journal of physiology. Regulatory, integrative and comparative physiology 288 (2), R345-53. DOI: 10.1152/ajpregu.00454.2004.

69. Tsai C. J., Kim S. A., Chu G. (2007): *Cernunnos/XLF promotes the ligation of mismatched and noncohesive DNA ends.* In Proceedings of the National Academy of Sciences of the United States of America 104 (19), pp. 7851–7856. DOI: 10.1073/pnas.0702620104.

70. Werner C., Fürster T., Widmann T., Pöss J., Roggia C., Hanhoun M. et al. (2009): *Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall.* In Circulation 120 (24), pp. 2438–2447. DOI: 10.1161/CIRCULATIONAHA.109.861005.

71. Yoo S., Dynan W. S. (1999): *Geometry of a complex formed by double strand break repair proteins at a single DNA end. Recruitment of DNA-PKcs induces inward translocation of Ku protein.* In Nucleic acids research 27 (24), pp. 4679–4686.

72. Zaremba T., Oliński R. (2010): *Oksydacyjne uszkodzenia DNA — ich analiza oraz znaczenie kliniczne.* In Postępy Biochemii 2010 (2), pp. 124–138.

73. Zhang W., Xiao S., Ahn D. U. (2013): *Protein oxidation. Basic principles and implications for meat quality.* In Critical reviews in food science and nutrition 53 (11), pp. 1191–1201. DOI: 10.1080/10408398.2011.577540.

74. Zhang Z., Zhu L., Lin D., Chen F., Chen D. J., Chen Y. (2001): *The three-dimensional structure of the C-terminal DNA-binding domain of human Ku70.* In The Journal of biological chemistry 276 (41), pp. 38231–38236. DOI: 10.1074/jbc.M105238200.

75. Zhang Z., Hu W., Cano L., Lee T. D., Chen D. J., Chen Y. (2004): *Solution structure of the C-terminal domain of Ku80 suggests important sites for protein-protein interactions.* In Structure (London, England : 1993) 12 (3), pp. 495–502. DOI: 10.1016/j.str.2004.02.007.

76. Zou L., Elledge S. J. (2003): *Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes.* In Science (New York, N.Y.) 300 (5625), pp. 1542–1548. DOI: 10.1126/science.1083430.