Network Pharmacology-Based Investigation of a Traditional Thai Longevity Formula for Anti-Aging Potential

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Abstract

Aging, considered by a progressive decline in physiological function, is a main contributor to disease and mortality. As a result, the study of anti-aging interventions remains a significant area of scientific investigation. Herbal formulations, particularly plant extracts, represent a promising avenue for modifying the aging development. This study investigates the anti-aging potential of a traditional Thai longevity formula, composed of *Boesenbergia rotunda*, *Curcuma zedoaria*, *Piper nigrum*, *Amomum krervanh*, and *Acorus calamus*, using a bioinformatics-based approach to elucidate its molecular mechanisms and pathways. The bioactive compounds in this formula extract were identified using High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS). These compounds were subjected to in silico analysis, including target prediction via the SwissTargetPrediction database. Functional and pathway enrichment analyses were conducted using Gene Ontology (GO) analysis and the ShinyGO 0.80 platform, while pathway visualization and mapping were performed using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Additionally, protein-protein interaction (PPI) analysis was carried out using the STITCH database. The analysis revealed that the bioactive compounds in the extract are associated with multiple key pathways, including steroid hormone biosynthesis, cancer-related signaling, cell survival and apoptosis, metabolic regulation, stress response, immune function, antioxidant defense, and pathways related to aging and longevity. These findings provide valuable insights into the molecular mechanisms underlying the potential anti-aging effects of this traditional herbal formula. The results support the hypothesis that the bioactive compounds target multiple longevity-associated pathways, positioning this formulation as a promising candidate for further investigation as a natural anti-aging therapeutic agent.

Keywords: Anti-aging / Traditional Thai Medicine / Gene Ontology / Pathway Enrichment Analysis / Protein-protein Interaction (PPI)

1. Introduction

Aging is a complex biological process involving multiple factors, characterized by a steady deterioration of physiological integrity, resulting in impaired function and increased susceptibility to mortality. It is a primary risk factor for numerous chronic diseases, including cardiovascular disorders, neurodegenerative conditions, metabolic syndromes, and cancer [1]. The biological hallmarks of aging include genomic instability, telomere attrition, epigenetic alterations, loss of proteostasis, mitochondrial dysfunction, cellular senescence, and disrupted intercellular communication [1][2].

Due to the significant health implications of aging, there has been an increasing focus on identifying strategies to postpone or alleviate age-related deterioration. Among these strategies, natural products, particularly medicinal herbs, have demonstrated potential due to their antioxidant, anti-inflammatory, and cellular protective properties [3]. Traditional Thai medicine, which encompasses centuries of practical knowledge, includes several herbal formulations believed to promote longevity and vitality [4].

Recent research indicate that polyherbal formulations from Traditional Thai medicine may exhibit anti-aging activity, including lifespan extension and stress resistance in models such as *Caenorhabditis elegans* [5]. These effects are largely attributed to phytochemicals, including flavonoids, terpenoids, and phenolic compounds, which interact with molecular pathways related to aging, such as oxidative stress modulation and apoptosis [6]. However, as the effects of the active compounds in this formula on biological mechanisms have not been studied, the current study employed bioinformatics approaches to elucidate and understand the effects of the active compounds in the formula on biological mechanisms, including compound target prediction (e.g., SwissTargetPrediction), gene ontology (GO) enrichment, KEGG pathway mapping and protein interaction (PPI) network analysis. Combining these computational tools with phytochemical profiling methods, such as high-performance liquid chromatography (HPLC) and gas chromatography-mass spectrometry (GC-MS), can help elucidate the pharmacological activities of the herbal formulations [7][8].

2. Objective

To elucidate the molecular mechanisms underlying the anti-aging effects of herbal formulas through a bioinformatics-based approach aimed at identifying key pathways and targets associated with their therapeutic action.

3. Scope of study

This study aims to investigate the anti-aging potential of a traditional Thai longevity formula. The research focused on following:

1. Phytochemical analysis to find beneficial substances utilizing HPLC and GC-MS techniques

2. Investigation of molecular mechanisms with bioinformatics methods including SwissTargetPrediction, Gene Ontology (GO), and Pathway Enrichment Analysis.

3. Identification of targets associated to age, focusing on biological impacts concerning oxidative stress, inflammation, and cellular senescence.

4. Materials and Methods

4.1 Preparation of Herbal Extract and Phytochemical Analysis

The traditional Thai longevity formula, consisting of *B. rotunda, C. zedoaria, P. nigrum, A. krervanh,* and *A. calamus*, was prepared by cleaning, drying, and grinding all plant materials into fine powders. The herbs were extracted with 80% ethanol by maceration at 25 °C for 72 hours. After vacuum filtration, the solvent was removed under reduced pressure using a rotary evaporator, followed by freeze-drying to obtain the final crude extracts for analysis. Phytochemical profiling of the herbal extracts was performed using High-Performance Liquid Chromatography (HPLC) and Gas Chromatography-Mass Spectrometry (GC-MS).

4.2 Target Prediction

The compounds were subjected to target prediction using the SwissTargetPrediction database, which predicts possible molecular targets based on the chemical structures of the compounds [9]. Targets were chosen if their likelihood score was higher than 0.1.

4.3 Gene Ontology (GO) Enrichment and Pathway Enrichment Analysis

The predicted targets were analyzed for Gene Ontology (GO) enrichment using the ShinyGO 0.80 platform. This analysis identifies key biological processes, molecular functions, and cellular components associated with the targets [10]. The molecular pathways affected by the herbal compounds were mapped using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database. This analysis identifies biological pathways related to aging, such as oxidative stress, apoptosis, and cell cycle regulation [8]. Protein-protein interactions among the predicted targets were explored using the STITCH database [11].

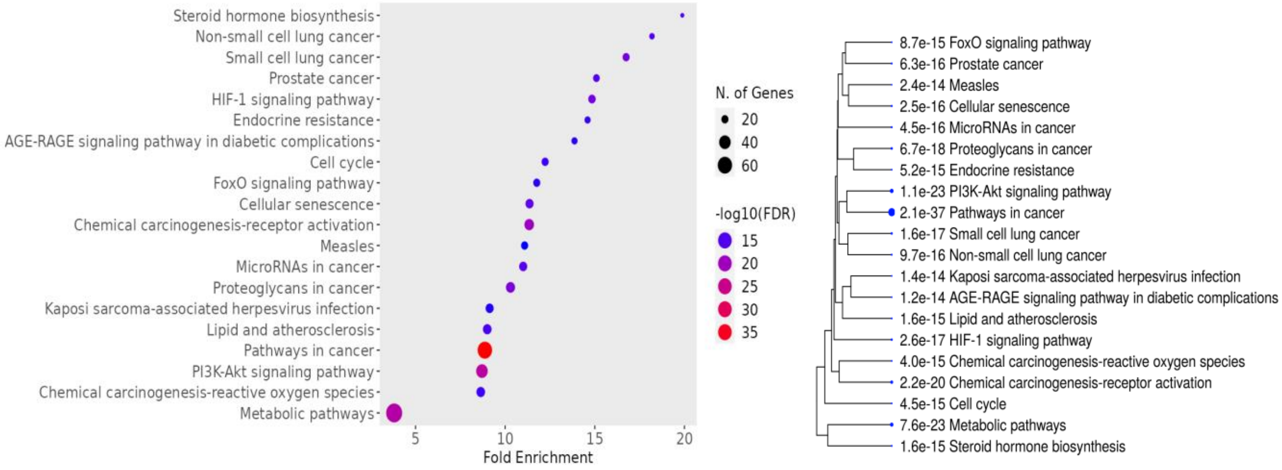
5. Result and discussion

5.1 Gene Ontology (GO) Enrichment Analysis

KEGG pathway enrichment analysis was conducted to explore the biological significance of the predicted target proteins of the traditional Thai longevity formula. As shown in Figure 1A, the top enriched pathways included steroid hormone biosynthesis, non-small cell lung cancer, small cell lung cancer, prostate cancer, and the HIF-1 signaling pathway. Notably, several pathways associated with metabolic regulation, cell cycle control, inflammation, and aging were also significantly enriched, such as the AGE-RAGE signaling pathway in diabetic complications, cellular senescence, FoxO signaling, and PI3K-Akt signalling pathway [12]. These findings suggest that the bioactive compounds may influence diverse biological processes relevant to aging, oxidative stress, cancer, and metabolic dysfunction.

The bubble plot illustrates that many of these pathways are statistically significant ($-\log10(\mathrm{FDR}) > 15$) and involve a substantial number of target genes, with several pathways showing over 60 gene associations. Pathways such as cellular senescence, FoxO signaling, and PI3K-Akt signaling are particularly relevant to anti-aging mechanisms, reflecting the formula's potential to modulate longevity-associated biological functions.

Hierarchical clustering of the enriched pathways (Figure 1B) revealed functional groupings, with aging-related, cancer-related, and metabolic pathways forming distinct clusters. Notable clusters included pathways related to cellular senescence, FoxO signaling, and AGE-RAGE signaling, highlighting the interconnected nature of aging, inflammation, and chronic disease. These results further support the hypothesis that this traditional Thai herbal formula exerts its potential anti-aging effects through multi-target modulation of key signaling networks.



(A) Biological pathways associated with biological compounds

(B) Relationship between biological pathways associated with biological compounds

Figure 1 shows (A) Biological pathways associated with biological compounds and (B) Relationship between biological pathways associated with biological compounds

5.2 Pathway Enrichment Analysis Of Targets Related To Antioxidant and Anti-Aging

Pathway enrichment analysis highlights the formula's potential antioxidant and anti-aging effects, it was found that among all the found pathways, the pathway most related to antioxidant and anti-aging activity is the FoxO signaling pathway because FoxO helps in stimulating antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). In addition, FoxO is also involved in DNA repair, cell division control, and apoptosis, which all help slow down the deterioration of the body and extend the lifespan of the organism

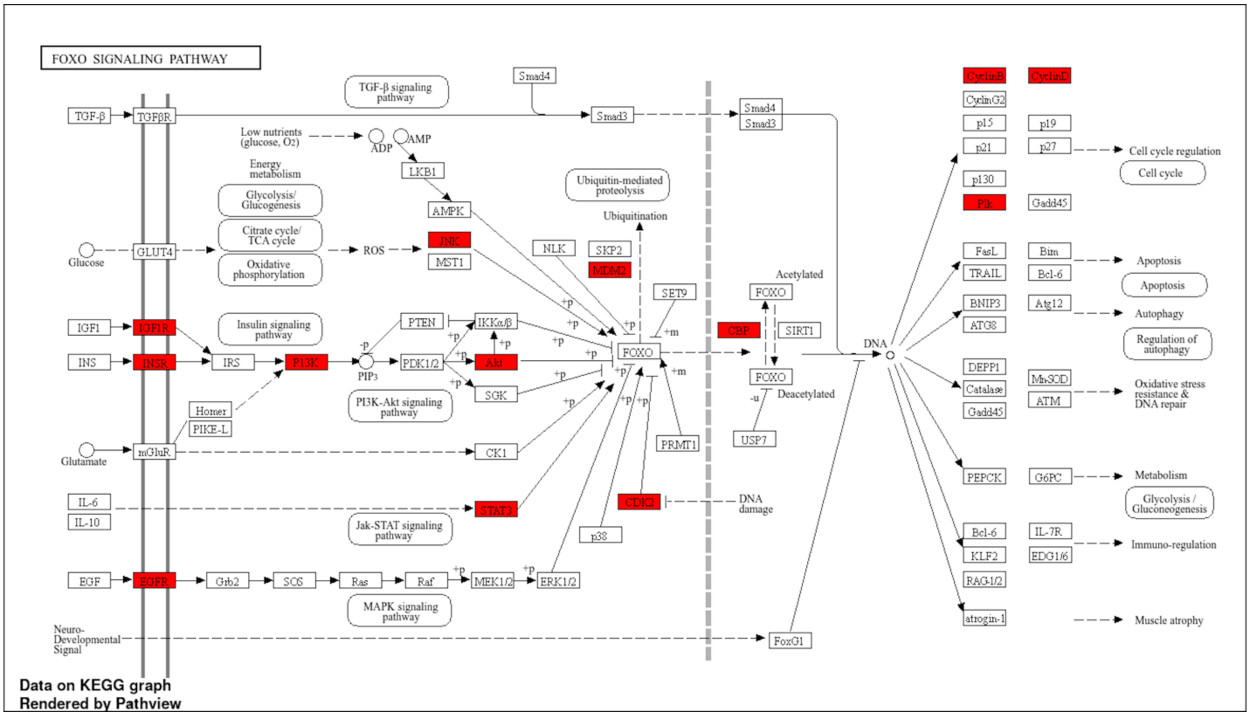


Figure 2 The pathway mapping of molecular targets associated with antioxidant and anti-aging activities of the Thai longevity formula, highlighting the involvement of the FoxO signaling pathway.

5.3 The protein-protein interaction (PPI) network analysis

PPI network analysis (Figure 3) reveals complex interactions among proteins involved in antioxidant and anti-aging pathways. Key genes identified include NQO2, EGLN1, and members of the AKR and HSD17B families, which play roles in detoxification, redox balance, and hormone regulation. The presence of tempo, a superoxide dismutase mimetic, further supports the formula’s potential in reducing oxidative stress. These findings underscore the formula’s multi-target, multi-pathway effects, aligning with systems pharmacology principles in traditional medicine.

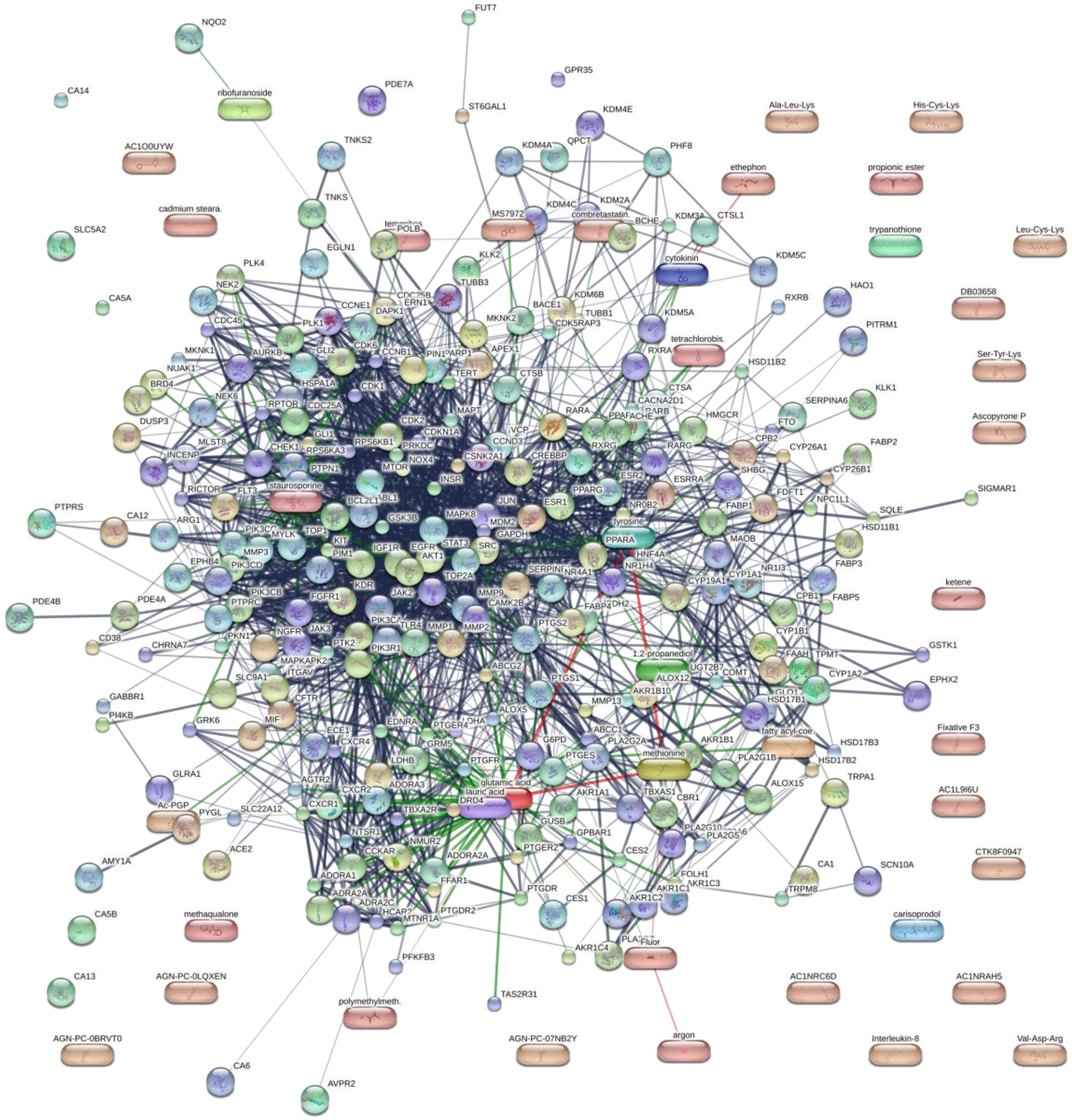


Figure 3 shows Protein-protein interaction (PPI) network analysis of the predicted targets of Predicted Targets from the Thai longevity formula.

5.4 Discussion

GO enrichment analysis indicated that the targets of the Thai longevity formula are significantly associated with biological processes such as cell signalling, metabolism, and responses to oxidative stress, all of which are central to aging and chronic disease development. The prominence of Metabolic pathways with high fold enrichment and gene representation reflects the importance of metabolic regulation in aging and disease states. Metabolic dysregulation is a well-established hallmark of aging and is intricately linked to age-related diseases including diabetes, cardiovascular disease, and cancer [1].

Moreover, the identification of the PI3 K-Akt signalling pathway and Steroid hormone biosynthesis pathways suggests potential roles in cell growth, survival, and hormonal regulation, which are all vital in maintaining homeostasis during aging [13]. The statistical significance and gene coverage within these pathways reinforce their relevance to the observed biological effects of the formula. Figure 1B highlights the interrelatedness of various disease and aging-related pathways. For example, small cell lung cancer and non-small cell lung cancer pathways suggest shared oncogenic processes, while pathways such as chemical carcinogenesis—reactive oxygen species and chemical carcinogenesis—receptor activation underscore the role of oxidative damage and receptor-mediated signalling in cancer development [14].

The connection between the FoxO signalling pathway and cellular senescence further supports the formula's anti-aging potential. FoxO transcription factors are known regulators of oxidative stress resistance, longevity, and metabolic balance [15]. Cellular senescence, although a protective mechanism against cancer, contributes to aging through the secretion of pro-inflammatory cytokines and matrix-degrading enzymes, commonly referred to as the senescence-associated secretory phenotype (SASP) [16].

Pathway enrichment analysis revealed that the pathway most involved in antioxidant and anti-aging activity is the FoxO signaling pathway, which plays a key role in the activation of antioxidant enzymes and the regulation of cell death and DNA repair, thus being a key mechanism involved in lifespan extension and maintenance of biosystem homeostasis in the body [17][18].

The PPI network (Figure 3) revealed a complex interplay between target proteins and related bioactive compounds. Key antioxidant and anti-aging-related genes such as NQO2, EGLN1, and members of the AKR1B and HSD17B families were identified. These genes are involved in detoxification, redox homeostasis, and steroid metabolism—processes that are essential for maintaining cellular integrity under stress [19].

Notably, the antioxidant tempo emerged as a significant node in the network. Tempo is known to mimic superoxide dismutase activity and has been studied for its protective effects against oxidative damage and inflammation [20]. This finding further supports the hypothesis that the Thai longevity formula exerts protective cellular effects through free radical scavenging and redox regulation mechanisms.

6. Conclusion

The integrative bioinformatics analysis of the Thai longevity formula revealed its potential involvement in modulating key biological processes related to aging, oxidative stress, inflammation, and chronic disease progression. This is consistent with the multifaceted nature of traditional medicine, which often targets a network of pathways rather than a single molecular mechanism. Taken together, these findings provide scientific support for the traditional use of the Thai longevity formula in promoting health and longevity. The multi-target nature of the formula is consistent with a systems biology perspective, where modulation of interconnected pathways may yield synergistic therapeutic effects. Future in vitro and in vivo validation of the identified targets and pathways is warranted to confirm these bioinformatic predictions and elucidate the molecular basis of its anti-aging effects.

Reference

[1] Lopez-Otin, C., Blasco, M. A., Partridge, L., Serrano, M., & Kroemer, G. (2013). The hallmarks of aging. *Cell*, *153*(6), 1194–1217. https://doi.org/10.1016/j.cell.2013.05.039

[2] Kennedy, B. K., Berger, S. L., Brunet, A., Campisi, J., Cuervo, A. M., Epel, E. S., ... & Rando, T. A. (2014). Geroscience: Linking aging to chronic disease. *Cell*, *159*(4), 709–713. https://doi.org/10.1016/j.cell.2014.10.039

[3] Maher, P. (2020). The potential of flavonoids for the treatment of neurodegenerative diseases. *International Journal of Molecular Sciences*, *21*(15), 5556. https://doi.org/10.3390/ijms21155556

[4] Sotanaphun, U., Sripattanakul, N., & Jiratchariyakul, W. (2021). A review of Thai traditional medicine longevity formulations: An ethnopharmacological perspective. *Journal of Ethnopharmacology*, *274*, 114017. https://doi.org/10.1016/j.jep.2021.114017

[5] Jiratchariyakul, W., & Mahidol, C. (2022). Traditional Thai medicines: Molecular mechanisms and therapeutic potentials. *Asian Pacific Journal of Tropical Biomedicine*, 12(3), 125–134. https://doi.org/10.1016/j.apjtb.2022.03.002

[6] Saewan, N., & Jimtaisong, A. (2015). Natural products as photoprotective agents in sunscreen: Development, formulations, and mechanisms of action. *International Journal of Cosmetic Science*, *37*(4), 298–305. https://doi.org/10.1111/ics.12211

[7] Gfeller, D., Grosdidier, A., Wirth, M., Daina, A., Michielin, O., & Zoete, V. (2014). SwissTargetPrediction: A web server for target prediction of bioactive small molecules. *Nucleic Acids Research*, 42(W1), W32–W38. https://doi.org/10.1093/nar/gku293

[8] Kanehisa, M., & Goto, S. (2000). KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Research*, *28*(1), 27–30. https://doi.org/10.1093/nar/28.1.27

[9] Daina, A., Michielin, O., & Zoete, V. (2019). SwissTargetPrediction: A web server for target prediction of bioactive small molecules. *Nucleic Acids Research, 42*(W1), W32–W38. https://doi.org/10.1093/nar/gku293

[10] Ge, S., Lu, S., & Chen, H. (2020). ShinyGO: A tool for gene ontology analysis and pathway enrichment. *Biological Direct, 15*(1), 18. https://doi.org/10.1186/s13062-020-00272-z

[11] Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., & Bork, P. (2019). STITCH 5: Augmenting protein–chemical interaction networks with tissue and affinity information. *Nucleic Acids Research, 47*(D1), D618–D623. https://doi.org/10.1093/nar/gky1001

[12] Kenyon, C. J. (2010). The genetics of ageing. *Nature*, 464(7288), 504–512.

[13] Manning, B. D., & Toker, A. (2017). AKT/PKB signaling: Navigating the network. *Cell*, 169(3), 381–405. https://doi.org/10.1016/j.cell.2017.04.001

[14] Reuter, S., Gupta, S. C., Chaturvedi, M. M., & Aggarwal, B. B. (2010). Oxidative stress, inflammation, and cancer: How are they linked? *Free Radical Biology and Medicine*, 49(11), 1603–1616. https://doi.org/10.1016/j.freeradbiomed.2010.09.006

[15] Calnan, D. R., & Brunet, A. (2008). The FoxO code. *Oncogene*, 27(16), 2276–2288. https://doi.org/10.1038/onc.2008.21

[16] Campisi, J. (2013). Aging, cellular senescence, and cancer. *Annual Review of Physiology*, 75, 685–705. https://doi.org/10.1146/annurev-physiol-030212-183653

[17] Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current Biology*, *24*(10), R453–R462. https://doi.org/10.1016/j.cub.2014.03.034

[18] Martins, R., Lithgow, G. J., & Link, W. (2016). Long live FOXO: Unraveling the role of FOXO proteins in aging and longevity. *Aging Cell*, *15*(2), 196–207. https://doi.org/10.1111/acel.12427

[19] Raza, H., John, A., Brown, E. M., & Benedict, S. (2020). Role of aldo-keto reductases in the development of drug resistance. *Frontiers in Pharmacology*, 11, 569300. https://doi.org/10.3389/fphar.2020.569300

[20] Elchuri, S., Oberley, T. D., Qi, W., Eisenstein, R. S., Jackson Roberts, L., Van Remmen, H., Epstein, C. J., & Huang, T. T. (2005). CuZnSOD deficiency leads to persistent and widespread oxidative damage and hepatocarcinogenesis later in life. *Oncogene*, 24(3), 367–380. https://doi.org/10.1038/sj.onc.1208223