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Long term p38-α deficiency up-regulates antioxidant enzymes through compensatory NF-κB activation

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Abstract

p38α MAPK may function as a mediator of reactive oxygen species signaling and thus p38α is considered a sensor of oxidative stress. In liver-specific p38α knock-out (KO) adult mice we previously found glutathione depletion and down-regulation of antioxidant enzymes. Our aim was to assess the influence of long-term p38α deficiency on oxidative stress and on the regulation of antioxidant enzymes in liver of old mice. To this end, wild type or liver-specific KO mice after weaning, at 4-6 months of age, or at 24 months of age were used. Reduced glutathione (GSH) and oxidized glutathione levels were determined by mass spectrometry, gene expression of antioxidant enzymes was determined by RT-PCR, and induction of NRF-2 and PGC-1α as well as activation of NF-κB were assessed by western blotting. We report that GSH levels decreased upon aging only in liver of wild-type mice, but not in p38α KO mice. The mRNA expression of glutathione peroxidase, Cu-Zn superoxide dismutase, Mn-superoxide dismutase, and glutamate cysteine ligase was up-regulated in adult wild-type in comparison with mice after weaning, but their gene expression was down-regulated in old wild-type mice. Although the mRNA expression of glutathione peroxidase, Cu-Zn superoxide dismutase, Mn-superoxide dismutase, and glutamate cysteine ligase was down-regulated in adult KO mice vs KO mice after weaning, their gene expression was up-regulated in old KO mice. This up-regulation was not associated with nuclear translocation of NRF-2, which decreased upon aging in KO mice, nor with up-regulation of PGC-1α. However, phosphorylation of p65 was markedly increased in old KO mice as an index of NF-κB activation. In conclusion, long term deficiency of p38α in the liver causes compensatory activation of NFκB that is likely to be responsible for the up-regulation of antioxidant enzymes upon aging, independently of Nrf-2 and PGC-1α.

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Psychiatric disorders and omega-3 fatty acids

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Abstract

Psychiatric disorders, especially mood disorders in children and adolescent are serious problem of all over the world in the field of child and adolescent psychiatry. In the recent years mood disorders occur in the earlier age. The prevalence of major depression (MD) is about 1-2% in preadolescent children and 3-8% in adolescents. When the major depression is not treated there is a big risk of worsening of symptoms, risk of suicide and development of comorbid disorders. The quality of life of the patient and its family is decreasing in the whole view.

The molecular basis of major depression is not well known. The main pathomechanism of MD is in noradrenergic, serotonergic and dopaminergic pathway dysregulation, nutition factors, which can influence structure and metabolism of lipids. It was found decreased level of omega 3 fatty aids (FA), increased ratio of omega 6/omega 3 FA in the serum and in erythrocyte membrane.

It is supposed that the oxidative neuronal injury can be prevented by dietary supplementation of antioxidants and that membrane phospholipids can be repaired by dietary supplementation of fatty acids. Omega-3 fatty acids may also participate in modulation of membrane fluidity, which influences the transmission of neurotransmitters. The membrane fluidity is affected by the ratio of phospholipids to free cholesterol. In addition, activation of the inflammatory response was found in depressive patients through increased production of pro-inflammatory cytokines (IL-1b, IL-6, interferon gamma, TNF-alpha) and eicosanoids (prostaglandin E2) in blood and cerebrospinal fluid. This results in increased lipid peroxidation and degradation of polyunsaturated fatty acids, which may result in increased oxidative stress. Omega-3 fatty acids also stimulate anti-inflammatory cytokines (IL-10) or inhibit the cyclooxygenase, platelet aggregation and formation of eicosanoids. The potential molecular mechanisms will be discussed.

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Targeted mass spectrometry methods for detecting oxidative post-translational modifications

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Abstract

Oxidative post-translational modifications (oxPTMs) can alter the function of proteins, and are important in the redox regulation of cell behaviour. The most informative technique to detect and locate oxPTMs within proteins is mass spectrometry (MS). However, proteomic MS data are usually searched against theoretical databases using statistical search engines, and the occurrence of unspecified or multiple modifications, or other unexpected features, can lead to failure to detect the modifications and erroneous identifications of oxPTMs. We have developed a new approach for mining data from accurate mass instruments that allows multiple modifications to be examined. Accurate mass extracted ion chromatograms (XIC) for specific reporter ions from peptides containing oxPTMs were generated from standard LC-MSMS data acquired on a rapid-scanning high-resolution mass spectrometer (ABSciex 5600 Triple TOF). The method was tested using proteins from human plasma or isolated LDL. A variety of modifications including chlorotyrosine, nitrotyrosine, kynurenine, oxidation of lysine, and oxidized phospholipid adducts were detected. For example, the use of a reporter ion at 184.074 Da/e, corresponding to phosphocholine, was used to identify for the first time intact oxidized phosphatidylcholine adducts on LDL. In all cases the modifications were confirmed by manual sequencing. ApoB-100 containing oxidized lipid adducts was detected even in healthy human samples, as well as LDL from patients with chronic kidney disease. The accurate mass XIC method gave a lower false positive rate than normal database searching using statistical search engines, and identified more oxidatively modified peptides. A major advantage was that additional modifications could be searched after data collection, and multiple modifications on a single peptide identified. The oxPTMs present on albumin and ApoB-100