Effect of Oxidative Stress on Mitochondrial Dynamics

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Abstract

Mitochondrial dynamics are related to the mitochondrial function and cellular requirements in mammalian cells. Maintenance of this network demands an important balance between mitochondrial fusion and fission processes, and likewise allows cells to respond rapidly to metabolic needs that are essential for cellular function. Changes in mitochondrial morphology have been observed in various diseases such as peripheral neuropathies, bipolar disorder (BD) and some cancers. As such, the aim of this research is to understand the relationship between mitochondrial morphology and oxidative stress. This was undertaken using both bioinformatics and experimental approaches.

Bioinformatics helped in investigating how the expression of mitochondrial fission and fusion proteins changes between diseased and normal tissue. Using glioblastoma (GBM) as a model, the expression of nine mitochondrial proteins was studied at the RNA level using publicly available microarray data. Clear changes in the pattern of expression were observed between healthy tissue and GBM, after which a metanalysis performed on cancer datasets confirmed significantly decreased expression for the majority of these mitochondrial proteins in GBM. Similar investigations in lung, breast and ovarian cancer also found expression of mitochondrial proteins to be significantly altered; however, no clear patterns of change were detected to explain the basis for mitochondrial dysregulation. The data suggested global changes in the network rather than a shift in mitochondrial dynamics.

Oxidative stress also contributes to the pathophysiology of cancer. Prior studies have demonstrated that such stress may lead to mitochondrial fragmentation by causing an imbalance between fission and fusion processes. A series of experiments investigated whether manipulating mitochondrial dynamics by over-expressing mitochondrial morphology proteins, including ganglioside differentiation-associated protein 1 (GDAP1), mitochondrial fission protein 1 (Fis1) and mitofusin 2 (Mfn2), could protect against oxidative damage. After treating with oxidative agents, cell viability was assessed using the MTT assay, apoptosis was measured by fluorescent cytometry, and changes to mitochondrial morphology were analysed through fluorescent microscopy.

When glutamate was sought to induce oxidative stress, a dose-responsive decrease in cell survival for COS-7 cells was observed; in turn, cell survival increased with over-expression of fission proteins and the addition of N-acetylcysteine (NAC). Quantitative analysis of mitochondrial morphology then revealed a shift in the number of cells having fragmented mitochondria after glutamate exposure. This shift was also observed for the GDAP1 and Fis1 over-expressing cell lines but to a lesser extent, as their basic morphology was already largely fragmented; these cell lines were also observed to have higher levels of apoptosis. NAC treatment counteracted cell death but did not rescue the mitochondrial network or extent of apoptosis. Further, too, NAC is thought to act largely as a precursor to glutathione (GSH). Effect of GSH concentration was also investigated through use of ethacrynic acid (EA) and menadione, both of which can deplete GSH. As discovered, both drugs caused a dose-responsive decrease in cell survival for COS-7 cells. By contrast, a minimal survival advantage was observed in cell lines over-expressing GDAP1, Fis1 and Mfn2; although, slight increases in mitochondrial fragmentation were noted with drug treatment.

Although GDAP1 is expressed throughout the body, mutations in this protein only result in neurodegenerative disease. Two isoforms of GDAP1 (B and C) were identified and characterised to determine whether they might complement GDAP1 function. Over-expression in COS-7 confirmed mitochondrial localisation and demonstrated that GDAP1B and GDAP1C could induce mitochondrial fragmentation. They also revealed a similar profile to GDAP1A in protecting against oxidative stress after treatment with glutamate, menadione and EA. That said, further work is still required to understand cellular expression and the relationship between these isoforms, GDAP1A and other mitochondrial morphology proteins.

Overall, this research demonstrates that manipulating mitochondrial morphology to increase mitochondrial fragmentation can protect against oxidative stress. It also shows that alteration in GSH levels could affect cell survival, most likely through interaction with mitochondrial morphology proteins. Further research needs to be undertaken to understand the mechanisms behind cellular control of mitochondrial dynamics, as focused on preventing oxidative damage. This may provide novel targets for the treatment and prevention of cancers and neurodegenerative disease.

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