

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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Table of contents

Abstract.....	i
Certificate of Authorship of Thesis	iii
Acknowledgements.....	v
Table of contents	vii
List of Figures	xi
List of Tables	xvii
Chapter 1 Introduction	1
1.1 Mitochondria.....	1
1.1.1 Role of mitochondria in life	1
1.1.2 Dynamics of mitochondrial morphology	2
1.1.3 Effect of mitochondrial fusion and fission proteins in mitochondrial disease ..	11
1.1.4 Relationship between mitochondria and peroxisomes.....	15
1.2 Introduction to GDAP1.....	16
1.2.1 Structure of GDAP1 and cellular localisation.....	16
1.2.2 Mutations in GDAP1: Linkage of GDAP1 with CMT	18
1.2.3 Cellular roles for GDAP1	20
1.3 Effect of changes in GDAP1 on whole organisms	26
1.4 Effect of oxidative stress on mitochondrial morphology proteins	28
1.5 Aim of this study.....	31
Chapter 2 Materials and methods.....	33
2.1 Materials	33
2.2 Methods used.....	38
2.2.1 Cell culture techniques	38
2.2.2 Transfection of cells	40
2.2.3 MTT assay for cell viability	40
2.2.4 Microscopy.....	41
2.2.5 Molecular biology methods	42
2.2.6 Statistical analysis	43

Chapter 3	Studies of fission and fusion proteins: A bioinformatics analysis	45
3.1	Introduction.....	45
3.2	Methodology.....	47
3.2.1	GEO data mining & analysis	48
3.2.2	Oncomine data mining & analysis	49
3.3	Results	49
3.3.1	Expression of different fission and fusion proteins in normal tissues.....	50
3.3.2	Expression of fission and fusion proteins in brain and nerve diseases	56
3.3.3	Expression of mitochondrial fission and fusion proteins in human glial brain tumours	67
3.3.4	Expression of mitochondrial fission and fusion proteins in other cancers	82
3.4	Discussion.....	104
Chapter 4	Effect of oxidative stress on mitochondrial morphology proteins	111
4.1	Introduction.....	111
4.2	Methodology.....	115
4.2.1	Flow Cytometry assay	116
4.2.2	Data analysis	117
4.3	Results	117
4.3.1	Glutamate exposure affects cellular proliferation and results in cell death...117	
4.3.2	Effect of glutamate or NAC exposure on mitochondrial morphology.....134	
4.3.3	Effect of other oxidative stress inducing agents on cell viability	148
4.3.4	Effect of oxidative stress inducing agents on mitochondrial morphology	160
4.4	Discussion.....	170
Chapter 5	Functional characterisation of GDAP1 isoforms (B and C)	177
5.1	Introduction.....	177
5.2	Methods	178
5.2.1	Bioinformatic methods	178
5.2.2	Confirmation of GDAP1 isoforms form.....	178
5.2.3	RNA Purification and Reverse Transcription.....	179
5.3	Results	181

5.3.1	Bioinformatics analysis	181
5.3.2	Expression of GDAP1 transcripts	185
5.3.3	Localisation of GDAP1 Isoforms (B and C)	190
5.3.4	Effect of Oxidative stress on cells expressing with GDAP1 Isoforms (B and C) 197	
5.4	Discussion.....	213
Chapter 6	Discussion.....	217
6.1	Overview	217
6.2	Exploration of mitochondrial dynamics in cancer models.....	217
6.3	Exploration of mitochondrial protein in non-cancer models	220
6.4	Investigating the effect of oxidative stress on mitochondrial dynamics	220
6.5	Functional roles of GDAP1 isoforms	224
6.6	Limitations.....	224
6.7	Conclusions and future directions	226
Appendixes.....		227
Appendix 1: Abbreviations.....		229
Appendix 2: Plasmid maps.....		233
Appendix 3: Supplementary data for Chapter 3.....		235
Appendix 4: Supplementary data for Chapter 4.....		291
Appendix 5: Supplementary data for Chapter 5.....		317
Appendix 6: Conference Abstracts		331
References		335

List of Figures

Figure 1.1 Mitochondrial functional roles	2
Figure 1.2 Mitochondrial dynamics is a balance of fission and fusion processes	4
Figure 1.3 Amino acid alignment of GDAP1 protein.....	17
Figure 1.4 A model proposed to study GDAP1 function in calcium homeostasis.	24
Figure 1.5 Effect of ROS and fragmentation.	26
Figure 1.6 Schematic figure showing effect of biological molecules on cell injury.	29
Figure 3.1 Ratio of mRNA expression for mitochondrial fission and fusion proteins in 32 normal human tissues.....	56
Figure 3.2 The mRNA expression of mitochondrial proteins in brain tissues from HIV patients.	59
Figure 3.3 The mRNA expression of mitochondrial genes in brain tissues from HIV -1 associated neurocognitive disorders	64
Figure 3.4 The expression of mitochondrial proteins in bipolar patients: dorsolateral prefrontal cortex.....	66
Figure 3.5 The expression of mitochondrial proteins in bipolar patients: orbitofrontal cortex	67
Figure 3.6 Expression of mitochondrial proteins in the glioma-derived tumor tissue in the brain.	69
Figure 3.7 Radar chart showing the expression pattern for mitochondrial proteins.....	70
Figure 3.8 Radar charts shows the expression of mitochondrial protein: GDS 1813.....	71
Figure 3.9 mRNA expression level of mitochondrial proteins in normal brain and four different types of brain tumors.....	72

Figure 3.10 Expression of DNMT1L in primary and secondary brain tumors.....	73
Figure 3.11 Radar charts showing the expression of mitochondrial proteins.....	74
Figure 3.12 Summary of studies involved in mitochondrial proteins in glioblastoma.....	77
Figure 3.13 Forrest plot comparing mRNA expression for mitochondrial fission and fusion protein in glioblastoma and normal brain tissue.....	81
Figure 3.14 Summary of studies involved in mitochondrial proteins in breast cancers	83
Figure 3.15 Forrest plot comparing mRNA expression for mitochondrial fission and fusion proteins in breast cancers.....	88
Figure 3.16 Summary of studies involved in mitochondrial proteins in lung cancer	89
Figure 3.17 Forrest plot comparing mRNA expression for mitochondrial fission and fusion proteins in lung cancer compared to control group.....	94
Figure 3.18 Expression of mitochondrial proteins in ovarian cancer	96
Figure 3.19 Summary of studies involved in ovarian cancer	98
Figure 3.20 Forrest plot comparing mRNA expression for mitochondrial proteins in ovarian cancer.....	103
Figure 4.1 Our hypothetical model linking oxidative stress and mitochondrial fragmentation are linked.	114
Figure 4.2 Flow -diagram of the experiments undertaken.....	115
Figure 4.3 Impact of glutamate and NAC on cell survival.....	118
Figure 4.4 Impact of glutamate on cell survival.....	119
Figure 4.5 The effect of glutamate and NAC on cell death in Neuro-2a cells.	122
Figure 4.6 Relative cell survival after treatment with glutamate or NAC in GDAP1 transfected cell lines.....	124

Figure 4.7 Representative photograph showing apoptosis and necrosis in COS-7 cells after glutamate treatment	127
Figure 4.8 The effect of glutamate and NAC on cell death in GDAP1 transfected COS-7 cells.	128
Figure 4.9 Relative Cell survival on Mfn2 and Fis1 transfected cells after glutamate treatment.	130
Figure 4.10 The effects of glutamate on cell death in Mfn2 and Fis1 transfected COS-7 cells.	133
Figure 4.11 Morphological pattern of mitochondrial networks in COS-7 cells.	135
Figure 4.12 Mitochondrial morphologies pattern observed in COS-7. transfected with EGFP constructs.....	136
Figure 4.13 Representative confocal pictures of COS-7 cells transfected with EGFP (control).	137
Figure 4.14 Representative confocal pictures of COS-7 cells were transiently transfected with EGFP- GDAP1.....	138
Figure 4.15 Representative confocal pictures of COS-7 cells were transiently transfected with EGFP- Mfn2.	139
Figure 4.16 Representative confocal pictures of COS-7 cells were transiently transfected with EGFP- Fis1.....	140
Figure 4.17 Mitochondria Morphology in COS-7 transfected with GDAP1.....	142
Figure 4.18 Mitochondrial morphology observed in GDAP1 transfected COS-7 cells after glutamate and NAC treatment.	143
Figure 4.19 Mitochondria Morphology in COS-7 cells transfected with Mfn2 and Fis1.	145
Figure 4.20 Mitochondrial morphology pattern in Mfn2 and Fis1 cells after glutamate and NAC treatment.....	146

Figure 4.21 Relative cell survival after treatment with BSO and NAC in COS-7 and Neuro-2a.	148
Figure 4.22 Relative cell survivals after treatment with varying concentration of EA with presence and absence of NAC.	150
Figure 4.23 Relative cell survivals after treatment with varying concentrations of Menadione with presence and absence of NAC.	151
Figure 4.24 Relative cell survival after treatment with Varying concentrations of Rot with presence and absence of NAC.	153
Figure 4.25 Relative cell survivals after treatment with varying concentrations of DTT with presence and absence of NAC.	154
Figure 4.26 Relative cell survivals after treatment with varying concentrations of EA with presence and absence of NAC.	156
Figure 4.27 Relative Cell survival after treatment with of Mfn2 and Fis1 transfected cells	157
Figure 4.28 Relative cell survivals after treatment with varying concentrations of Menadione	158
Figure 4.29 Relative Cell survival after treatment with varying concentrations of Menadione	159
Figure 4.30 Mitochondria Morphology in COS-7 transfected with EGFP and treated with EA and Menadione.....	161
Figure 4.31 Mitochondria Morphology in COS-7 transfected with EGFP GDAP1 and treated with EA and Menadione.....	162
Figure 4.32 Mitochondrial morphologies pattern observed in GDAP1A cells after EA and Menadione with NAC treatment.	163
Figure 4.33 Mitochondria Morphology in COS-7 transfected with Mfn2 and Fis1 treated with EA plus or minus NAC.....	166

Figure 4.34 Mitochondria Morphology in COS-7 transfected with Mfn2 and Fis1 treated with Menadione.....	167
Figure 4.35 Mitochondrial morphologies pattern observed in Mfn2 and Fis1 cells treated	169
Figure 5.1 5' transcript for alternate splicing of GDAP1.....	182
Figure 5.2 Agarose gel showing amplification product for GDAP1 isoform 5'RACE.	187
Figure 5.3 Agarose gel showing bands of different cell lines for isoform	189
Figure 5.4 Representative confocal microscope images of COS-7 cells that were transiently transfected with EGFP GDAP1A.....	191
Figure 5.5 Representative confocal microscope images of COS-7 cells transiently transfected with EGFP GDAP1B.....	192
Figure 5.6 Representative confocal microscope images of COS-7 cells transiently transfected with EGFP GDAP1C.....	193
Figure 5.7 Quantification of Mitochondria Morphology in COS-7 transfected with GDAP1 Isoforms	195
Figure 5.8 Mitochondrial morphologies pattern observed between control and GDAP1 isoforms transfected cells.....	196
Figure 5.9 Relative cell survival after treatment with glutamate and NAC concentration. ...	198
Figure 5.10 Mitochondria Morphology in COS-7 cells transfected with GDAP1B and GDAP1C.	201
Figure 5.11 Mitochondrial morphologies pattern observed isoforms with glutamate	202
Figure 5.12 Relative cell survivals after treatment of transfected cells with different concentrations of EA.....	204
Figure 5.13 Relative Cell survival after treatment of transfected cells with different concentrations of menadione.....	206

Figure 5.14 Mitochondria Morphology in COS-7 transfected with GDAP1B and GDAP1C and treated with EA208

Figure 5.15 Mitochondrial morphologies observed in GDAP1B and GDAP1C COS-7 cells after EA.209

Figure 5.16 Mitochondria Morphology in COS-7 transfected with GDAP1B and GDAP1C. ...211

Figure 5.17 Mitochondrial morphologies observed in GDAP1B and GDAP1C transfected COS-7 cells after Menadione212

List of Tables

Table 1-1 Metabolic effect of alterations in fission and fusion proteins on cell metabolism and proliferation	5
Table 1-2 Mitochondrial dynamics in human diseases.....	12
Table 1-3 Fission and Fusion protein alteration in Neurodegenerative diseases	13
Table 2-1 Chemicals and reagents	33
Table 2-2 Kits used for molecular methods	35
Table 2-3 Enzymes used in the restriction digestion of DNA	35
Table 2-4 Software used in analysis (Imaging, quantitative & statistical).....	35
Table 2-5 Equipment used in this research	36
Table 2-6 Cell lines used in this study	37
Table 2-7 Bacterial cultures & Plasmids.....	37
Table 2-8 Stains used in Microscopy.....	41
Table 3-1 List of public database websites used in the study	47
Table 3-2 Selection criteria used for GEO Profiles.....	48
Table 3-3 Microarray experiments showing the expression of mitochondrial fission and fusion proteins in healthy normal human tissues.	50
Table 3-4 Tissues with significantly altered protein expression	51
Table 3-5 Statistical analysis of expression levels in HIV patients with neurocognitive disorders for mitochondrial protein	58
Table 3-6 Statistical analysis of expression levels in HIV patients of mitochondrial fusion and fission protein	61

Table 3-7 The mRNA expression levels of different fusion and fission proteins in BD relative to normal controls.....	65
Table 3-8 Summary of mRNA expression of fission and fusion protein in human glial brain tumors (GDS 1962).....	68
Table 3-9 Summary of mRNA expression of fission and fusion protein human glial brain tumors (GDS 1813)	71
Table 3-10 Summary of mRNA expression level of fission and fusion protein in primary and secondary brain tumors (GDS4467).....	75
Table 3-11 Summary of studies investigating expression levels for fission and fusion protein in glioblastoma.....	76
Table 3-12 Summary of studies investigating expression levels for fission and fusion proteins in breast cancer.....	83
Table 3-13 Summary of studies investigating expression levels of different fission and fusion proteins when comparing in Lung adenocarcinoma	89
Table 3-14 The mRNA expression levels for different fusion and fission proteins in ovarian cancer cells.....	95
Table 3-15 Summary of studies involved in expression levels of different fission and fusion protein with ovarian cancer cells.....	97
Table 4-1 Effect of oxidative agents used on mitochondrial fission and fusion.....	113
Table 4-2 Chemicals used as oxidative stress agents in this chapter	116
Table 4-3 Statistical analysis of cell survival with glutamate or NAC treatment.....	119
Table 4-4 Relative cell survival on different NAC and glutamate concentration	120
Table 4-5 Percentage (%) of apoptosis and necrosis in the cell population.....	121
Table 4-6 Statistical analysis of cell survival with glutamate and NAC treatment.	125
Table 4-7 Percentage (%) of the cell population given as live, dead or apoptotic.....	126

Table 4-8 Statistical analysis of the cell population data on GDAP1 transfected cells.	126
Table 4-9 Statistical analysis on Mfn2 and Fis1 transfected cells after glutamate and NAC treatment.....	131
Table 4-10 Percentage (%) of the cell population given as live, dead or apoptotic after glutamate treatment.	132
Table 4-11 Statistical analysis of the cell population data on Mfn2 and Fis1 transfected cells.	132
Table 4-12 Statistical analysis of mitochondrial morphology for GDAP1 transfected cells. .	143
Table 4-13 Statistical analysis of mitochondrial morphology pattern for Mfn2 transfected cells	147
Table 4-14 Statistical analysis of mitochondrial morphology pattern for Fis1 transfected cells	147
Table 4-15 Statistical analysis of cell survival after treatment with BSO and/or NAC.	149
Table 4-16 Statistical analysis of cell survival with EA and NAC concentration	150
Table 4-17 Statistical analysis of cell survival with Menadione and NAC concentration.....	152
Table 4-18 Statistical analysis of cell survival with Rot and NAC concentration	153
Table 4-19 Statistical analysis of cell survival with DTT and NAC concentration.	154
Table 4-20 Statistical analysis of cell survival in GDAP1 transfected cell	156
Table 4-21 Statistical analysis of cell survival in GDAP1 transfected cells.	158
Table 4-22 Statistical analysis of mitochondrial morphology pattern with Menadione and NAC concentration.....	164
Table 5-1 Primers used in the rapid amplification of 5' cDNA ends (5'RACE)	179
Table 5-2 Cell lines used for RNA isolation	180
Table 5-3 Thermal cycler conditions for specific primer pairs.	181

Table 5-4 Variant transcripts identified in the NCBI database using BLAST	183
Table 5-5 Variant transcripts identified in the Ensembl database using BLAST	184
Table 5-6 Expression studies of GDAP1B and GDAP1C in tissue types	185
Table 5-7 mRNA expression analysis of GDAP1 isoforms in cell lines.	186
Table 5-8 Statistical analysis for quantification of mitochondrial morphology for Isoforms	196
Table 5-9 Statistical analysis of cell survival in GDAP1B and GDAP1C transfected cells treated with glutamate.....	199
Table 5-10 Statistical analysis of mitochondrial morphology pattern for GDAP1B with glutamate.....	203
Table 5-11 Statistical analysis of mitochondrial morphology pattern for GDAP1C with glutamate.....	203
Table 5-12 Statistical analysis of cell survival in GDAP1B and GDAP1C transfected cells treated with EA and NAC.	205
Table 5-13 Statistical analysis of cell survival in GDAP1B and GDAP1C transfected cells with Menadione and NAC.....	206