UNIVERSITI PUTRA MALAYSIA

INVESTIGATING INTERPLAY BETWEEN MITOCHONDRIAL DNA MUTATIONS, OXIDATIVE STRESS AND CELL DEATHS IN MALAYSIAN BREAST CANCER PATIENTS

RAEVATHI A/P OMASANGGAR

FPSK(m) 2021 7

INVESTIGATING INTERPLAY BETWEEN MITOCHONDRIAL DNA MUTATIONS, OXIDATIVE STRESS AND CELL DEATHS IN MALAYSIAN BREAST CANCER PATIENTS

By

RAEVATHI A/P OMASANGGAR

Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Master of Science

January 2021

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment of the requirement for the degree of Master of Science

INVESTIGATING INTERPLAY BETWEEN MITOCHONDRIAL DNA MUTATIONS, OXIDATIVE STRESS AND CELL DEATHS IN MALAYSIAN BREAST CANCER PATIENTS

By

RAEVATHI A/P OMASANGGAR

January 2021

Chairman : Sandra a/p Maniam, PhD
Faculty : Medicine and Health Sciences

Mitochondrial DNA (mtDNA) mutations, oxidative stress and resistance to cell deaths increase one's risk for breast cancer. This study aims to identify the types of mtDNA mutations, levels of protein expression of oxidative stress, apoptosis, autophagy and mitophagy in breast cancer as well as the association between mtDNA mutations and the levels of protein expression. Female breast cancer patients (n=20) without neoadjuvant treatment were recruited with informed consents. Samples were 20 matched breast tumours with corresponding normal breast tissues. The entire mtDNA (16.6 kb) was sequenced using next-generation sequencing (NGS) and the levels of protein expressions were studied using tissue microarray and immunohistochemistry. A total of 18 of 20 patients had at least one somatic mtDNA mutation in their tumour samples. Overall, 65 somatic mutations were identified, with 30 novel mutations. The majority (59%) of the somatic mutations were in the coding region, whereas only (11%) of the mutations occurred in the D-loop. Notably, (15.4%) of somatic mutations in the protein-coding regions were potentially deleterious. A total of 753 germline mutations were identified and four of which were novel mutations. In comparison to somatic mutations, <1% of germline mutations are harmful. Immunohistochemistry study showed inconsistent expressions of MnSOD2, LC3B, BNIP3 and Parkin in breast cancer tissues. The expression of Beclin-1 was consistently positive and the expression of CC3 was consistently negative in all breast cancer cases. The differences in Beclin-1 expression between cancer and matched normal tissues were significant ($p<0.001$). Investigation of the relationship between somatic mtDNA mutations and protein expression levels of MnSOD2, CC3, LC3B, Beclin-1, BNIP3 and Parkin showed no significant differences. The findings of this study may enhance the current knowledge of mitochondrial-regulated cell mechanisms in breast cancer in Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

MENGKAJI INTERAKSI ANTARA MUTASI DNA MITOKONDRIA, TEKANAN OKSIDATIF DAN KEMATIAN SEL DALAM KANSER PAYU DARA DI MALAYSIA

Oleh

RAEVATHI A/P OMASANGGAR

Januari 2021

Pengerusi : Sandra a/p Maniam, PhD
Fakulti : Perubatan dan Sains Kesihatan

Mutasi DNA mitokondria (mtDNA), tekanan oksidatif dan rintangan terhadap kematian sel meningkatkan risiko seseorang untuk kanser payudara. Kajian ini bertujuan untuk mengenalpasti jenis mutasi mtDNA dan tahap ekspresi protin MnSOD2, CC3, LC3B, Beclin-1, BNIP3 dan Parkin dalam kanser payudara serta menentukan perkaitan antara mutasi mtDNA dan tahap ekspresi protin. Pesakit kanser payudara wanita (n = 20) tanpa rawatan neoadjuvant direkrut dengan persetujuan yang dimaklumkan. Sampel adalah 20 tisu kanser payudara yang dipadankan dengan tisu payudara normal. Seluruh mtDNA (16.6 kb) dijujukan menggunakan penjujukan generasi kedua (NGS) dan tahap ekspresi protin dikaji menggunakan immunohistokimia. Sebanyak 18 daripada 20 sampel tisu kanser payudara mempunyai sekurang-kurangnya satu mutasi mtDNA somatik. Secara keseluruhan, 65 mutasi somatik dikenalpasti, dengan 30 mutasi novel. Majoriti (59%) daripada mutasi somatik adalah di gen pengekodan, manakala hanya (11%) daripada mutasi somatik berlaku dalam gelung D. Sebanyak (15.4%) mutasi mtDNA mempunyai potensi untuk bersifat patogenik. Di samping itu, 753 mutasi adalah diwarisi dan empat daripadanya adalah mutasi novel. Berbanding mutasi somatik, <1% daripada mutasi warisan adalah berbahaya untuk kefungsian sel. Kajian immunohistokima menunjukkan ekspresi MnSOD2, CC3, LC3B, BNIP3 dan Parkin yang tidak konsisten dalam semua sampel kanser payudara dan normal payudara. Tahap ekspresi protin Beclin-1 adalah positif dan konsisten dalam semua tisu kanser payudara. Perbezaan ekspresi protin Beclin-1 dalam tisu kanser dan normal payudara adalah signifikan pada aras $p<0.001$. Penyiasatan hubungan antara mutasi mtDNA yang somatik dan tahap ekspresi protin MnSOD2, CC3, LC3B, Beclin-1, BNIP3 dan Parkin tidak menunjukkan perbezaan yang ketara. Hasil kajian ini dapat meningkatkan pengetahuan terkini mengenai mekanisme sel yang diatur mitokondria dalam barah payudara di Malaysia.

ACKNOWLEDGEMENTS

The completion of this research study involved people from various backgrounds whom I would like to acknowledge as a form of appreciation in this thesis. First and foremost, I would like to express my sincerest gratitude to my parents and brother, Mr Omasanggar Venkidasalam, Mrs Saraswathi Sadayen and Mr Nithiyan Omasanggar for their endless financial and emotional support towards the completion of this study. Their unending understanding and patience gave me the courage and determination to face all the challenges that came throughout the journey to complete my research.

I would also like to thank and express my gratitude to the chairperson of the study, Dr Sandra Maniam for the funding, supervision and guidance offered throughout the research. My sincere appreciation to co-supervisor Prof. Madya Dr Maizaton Atmadini Abdullah for her generous guidance and support in completing the study. In addition to her role as a pathologist for the study, Prof. Madya Dr Maizaton contributed to the clinical sample and data collections. A special thanks to Dr Hasni Idayu Saidi from Department of Biomedical Sciences for statistical consultations offered to support the findings of the study.

This study would not have started without groups of health professionals led by Dr Nor Aina Emran from Hospital Kuala Lumpur, Dr Atiki Falparado Ahmad from National Cancer Institute and Dr Normayah Kitan from Hospital Putrajaya. Teams of breast surgeons, medical officers, pathologists, ward staff, operating theatre staff, nurses, science officers, research officers and medical laboratory technologists together helped me on the journey of patient recruitment, collection of clinical breast cancer samples and collection of clinical data. Hereby, a million thanks to all of them for having and guiding me with full support for the study.

I would also like to express my deepest respect and sincerest gratitude to the Director and Deputy Director of Integrative Pharmacogenomics Institute (iPROMISE), UiTM Puncak Alam Campus, Prof. Dato' Dr Mohd Zaki Salleh and Prof. Dr Teh Lay Kek, as well as to research fellows, Dr Ang Geik Yong and Dr Yu Choo Yee. They offered facilities and technical supports in next-generation sequencing (NGS) to meet the aims of this study. Besides, their encouragement during my attachment has always been motivating. I also take this opportunity to thank the rest of the iPROMISE staff and students for sharing the values of research and teamwork throughout my research attachment period.

This study also involved a brief attachment period with Ms Nur Maya Sabrina Tizen, Research Officer and Dr Suria Hayati, Research Investigator at the Universiti Kebangsaan Malaysia Medical Centre (UKMMC) to construct microarray of breast cancer tissues. I am very grateful for their generous support in giving me a full priority, cooperation and guidance to complete the construction of microarrays of breast cancer tissues within a very short duration.

I would also like to express my utmost gratitude to all the science officers and laboratory staff of the Department of Human Anatomy, Faculty of Medicine and Health Sciences, UPM and the Department of Pathology, Faculty of Medicine and Health Sciences, UPM for giving me space and assistance during the research study.

Lastly, a special appreciation for all the volunteered female breast cancer patients. Every patient I recruited for the study taught me a lot of life lessons to go with me forever. Even though I only studied the cases of 20 breast cancer patients for my master's degree, I was involved in the recruitment of about 100 female breast cancer patients. Apart from receiving medical treatments, the spirits they hold faithfully in their heart to fight the disease and survive are the living messages in my life.

I certify that a Thesis Examination Committee has met on 12 January 2021 to conduct the final examination of Raevathi a/p Omasanggar on her thesis entitled "Investigating Interplay between Mitochondrial DNA Mutations, Oxidative Stress and Cell Deaths in Malaysian Breast Cancer Patients" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded Master of Science.

Members of the Thesis Examination Committee were as follows:

Suhaili Binti Abu Bakar @ Jamaludin, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Norhafizah Binti Mohtarrudin, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Internal Examiner)

Norfilza Mohd Mokhtar, PhD
Professor
Faculty of Medicine
National University of Malaysia
Malaysia
(External Examiner)

ZURIATI AHMAD ZUKARNAIN, PhD
Professor Ts. and Deputy Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 27 April 2021

This thesis was submitted to the Senate of Universiti Putra Malaysia and has been accepted as fulfilment of the requirement for the degree of Master of Science. The members of the Supervisory Committee were as follows:

Sandra a/p Maniam, PhD
Senior Lecturer
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Chairman)

Maizaton Atmadini binti Abdullah, MBBS, MPath, PhD
Associate Professor
Faculty of Medicine and Health Sciences
Universiti Putra Malaysia
(Member)

ZALILAH MOHD SHARIFF, PhD
Professor and Dean
School of Graduate Studies
Universiti Putra Malaysia

Date: 06 May 2021

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABC	Avidin-biotin complex
ASR	Age-standardized rate
Atg	Autophagy-related protein
ATM	Amplicon tagment mix
ATP	Adenosine triphosphate
BCS	Breast conserving surgery
BMI	Body mass index
BNIP3	BCL2/adenovirus E1B 19kDa protein-interacting protein 3
Bp	Base pair
bPCR	Bridge PCR
CC3	Cleaved caspase 3
CCD	Charged coupled device
cDNA	Complementary deoxyribonucleic acid
COI	Cytochrome c oxidase I
COII	Cytochrome c oxidase II
COIII	Cytochrome c oxidase III
CYB	Cytochrome b
DCIS	Ductal carcinoma in-situ
D-Loop	Displacement loop
ddNTPs	Dideoxynucleotides triphosphates
DNA	Deoxyribonucleic acid
dsDNA	Double-stranded deoxyribonucleic acid
ER	Estrogen receptor

ETC	Electron transport chain
FASTQ	Text based format quality scores
FI-NTPs	Fluorescently labelled NTPs
FISH	Fluorescence in situ hybridization
FNAC	Fine-needle aspiration cytology
H&E	Haematoxylin and eosin staining
HER2	Human epidermal growth factor receptor 2
HVR-1	Hypervariable region 1
HVR-2	Hypervariable region 2
IDC	Invasive ductal carcinoma
IHC	Immunohistochemistry
ILC	Invasive lobular carcinoma
Indel	Insertion and deletion
ISH	In situ hybridization
JKEUPM	Jawatankuasa Etika Universiti Putra Malaysia
LC3B	Light chain 3 isoform B
LCIS	Lobular carcinoma in situ
LR-PCR	Long-range polymerase chain reaction
MnSOD2	Manganase superoxide dismutase 2
mtDNA	Mitochondrial DNA
NaOH	Sodium hydroxide
ND1	NADH-ubiquinone oxidoreductase chain 1
ND2	NADH dehydrogenase 2
ND3	NADH dehydrogenase 3
ND4	NADH dehydrogenase 4

ND4L	NADH-ubiquinone oxidoreductase chain 4L
ND5	NADH dehydrogenase 5
ND6	NADH dehydrogenase 6
nDNA	Nuclear DNA
NGS	Next generation sequencing
NPM	Nextera PCR master mix
OXPHOS	Oxidative phosphorylation
PCR	Polymerase chain reaction
PR	Progesteron receptor
rCRS	Revised cambridge reference sequence
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
RTA	Real-time analysis
SAV	Sequencing analysis viewer
SBS	Sequencing-by-synthesis
SNPs	Single nucleotide polymorphisms
TD	Tagment DNA buffer
TMA	Tissue microarray
tRNA	Transfer ribonucleic acid
UPM	Universiti Putra Malaysia
UKMMC	Universiti Kebangsaan Malaysia Medical Centre
VCF	Variant call format
WES	Whole exome sequencing
WGS	Whole genome sequencing

CHAPTER 1

INTRODUCTION

1.1 Research Background

Cancer of the breast is the most common malignancy in women, accounting for 2.1 million incidents worldwide (Bray et al., 2018). An excess of 626, 679 patients died from this disease in 2018 (Bray et al., 2018). The malignancy is also the most common disease reported among Malaysians (Fitzmaurice et al., 2017). In 2016, the Department of Statistics reported an increase in deaths due to breast neoplasms and it remains one of the leading causes of cancer fatality in women in Malaysia (Department of Statistics Malaysia, 2017) despite recent advances in diagnosis, prognosis and treatment.

Breast cancer is a disease where abnormal cells develop through the epithelial cells of the breast ducts and breast lobules. Breast cancer occurs in both sexes. However, a clear majority of the incidence occurred in women and the elderly, where 1 in 27 women are estimated to be at risk of developing breast cancer by the age of 65 years and older (Azizah et al., 2019). The incidence of female breast cancer in Malaysia varies by age and ethnicity. To date, the National Cancer Registry 2012-2016 reported the highest age-standardized rate (ASR) incidence in Chinese (40.7 per 100, 000) followed by Indians (38.1 per 100, 000) and Malays (31.5 per 100, 000) (Azizah et al., 2019).

The development of cancer is related to diverse genetic mutations found in both nuclear and mitochondrial genomes. Somatic alteration in the mitochondrial genome has recently been recognized as a contributing factor in the tumorigenesis of various cancers, including breast cancer (Yadav & Chandra, 2013). The mitochondrial genome or mitochondrial DNA (mtDNA) is found within mitochondria, a cytosolic organelle in eukaryotic cells. The mtDNA consists of a double-stranded DNA loop of 16, 659 base pairs, which is present in thousands of copies in cells. It has 37 genes comprise of 13 polypeptides of the electron transport chain (ETC), 22 transfer RNAs (tRNAs) and 2 ribosomal RNAs (rRNAs) for protein synthesis and translation within the mitochondria (Chinnery & Hudson, 2013).

In comparison to nuclear DNA (nDNA), mtDNA is susceptible to higher mutation rate due to its proximity to reactive oxygen species (ROS) generated as byproducts of oxidative phosphorylation (OXPHOS) (Yadav & Chandra, 2013). The lack of protective histones and DNA repair mechanisms further reduces the recovery capacity of mtDNA (Yadav & Chandra, 2013). This natural phenomenon in mitochondria is one of the contributing factors in the development of cancer. The ROS molecules generated at low levels in the OXPHOS cycle become signalling molecules in activating oncogenic signalling pathways and accumulate oncogenic mtDNA defects when the ROS levels are elevated (Porporato et al., 2017). MtDNA mutations have thus been implicated in

carcinogenesis and frequently observed in all types of human cancer. Somatic mtDNA mutations are detected in about 60% of breast cancers and are identified as reliable indicators for breast cancer prevention and future therapeutic applications (Yadav & Chandra, 2013; Li et al., 2016).

Manganese superoxide dismutase 2 (MnSOD2) is a major antioxidant protein detoxifying ROS endogenously in the mitochondria (Snezhkina et al., 2019). MnSOD2 is encoded by the superoxide dismutase (SOD2) nuclear gene (locus 6q25.3) and localized in the mitochondria (Church et al., 1992). The MnSOD2 is well known to have altered expression in various cancers including breast cancer, where it is more frequently elevated in cancer cells compared to normal cells (Becuwe et al., 2014; Borrelli et al., 2014). Higher levels of MnSOD2 were significantly associated with increased cancer aggressiveness and metastatic potential with poorer prognosis and lower survival rates (Kumar et al., 2014). Besides, MnSOD2 has also previously been reported to play tumour suppressor activity that inhibits cell proliferation and intensifies apoptosis in the early stages of cancer (Oberley, 2005). Recently, MnSOD2 has been identified as a potential biomarker in the identification of oxidative stress, metastatic cancer cells as well as a therapeutic tool in combating aggressive breast cancer (Fu et al., 2016).

Autophagy serves as an oxidative stress regulator, eliminates oxidatively damaged proteins and organelles as well as the cellular sources responsible for excessive ROS production (Lee et al., 2012). Autophagy is also known as an alternative metabolic pathway for tumour cell survival (Roy & Debnath, 2010). In particular, autophagy of mitochondria also known as mitophagy is recognized for mitochondrial turnover (Lee et al., 2012). Mitophagy is also seen as an essential process in the mitochondria to regulate uncontrolled oxidative stress (Lee et al., 2012). In a similar line, regulation of oxidative stress levels in cancer cells is known to impact the apoptotic process in cancer cells (Favaloro et al., 2012). Therefore, the key mediators of cell deaths such as cleaved caspase-3 (CC3) (apoptotic pathway), Beclin-1 and Light Chain (LC) 3B, (autophagy pathway) and BNIP3 and Parkin (mitophagy pathway) are commonly studied to understand the relationship between expression of these markers, cell death response and breast cancer prognosis.

The present study uses next-generation sequencing (NGS) to examine mtDNA alterations in 20 breast cancer patients. Besides, the oxidative stress scavenger protein and the expression of cell death mediator proteins in apoptosis, autophagy and mitophagy were assessed immunohistochemically. The findings from this study will dissect the central role of mitochondria in breast cancer. The knowledge gained will also inspire the identification of new cancer biomarkers and treatment of breast cancer.

1.2 Problem Statement

Mitochondrial regulated activities such as mtDNA mutations, scavenging of oxidative stress by MnSOD2, expression of CC3 in apoptosis, expression of Beclin-1 and LC3B protein in autophagy and expression of BNIP3 and Parkin in mitophagy have been

reported as crucial factors in the development of breast cancer. Numerous studies have documented mtDNA mutations in cancers, but no study was conducted to document mutations in the entire mtDNA (16.6 kb) in fresh breast cancer specimens in Malaysia. Identification of mtDNA genetic marker in breast cancer needs to be addressed. The expression of CC3 was found to increase after neoadjuvant treatments to cause apoptosis and tumour cell repopulation. Fewer clinical studies reported the expression of CC3 including MnSOD2, Beclin-1, LC3B, BNIP3 and Parkin in primary breast cancer without neoadjuvant treatments. It is crucial to understand the function of CC3, MnSOD2, Beclin-1, LC3B, BNIP3 and Parkin in the normal physiology of breast cancer development. The mutations in mtDNA were found to affect the regulation of oxidative stress, apoptosis, autophagy and mitophagy. However, the relationship between mtDNA mutations and expressions of MnSOD2, CC3, Beclin-1, LC3B, BNIP3 and Parkin has not been clarified and it is still unclear which one of these is dominant in breast carcinogenesis.

1.3 Study Justification

Breast cancer is the most often occurring cancer in women worldwide and predicted to reach approximately 3.2 million new cases per year by 2050 (Tao et al., 2015). In Malaysia, breast cancer is also the major public health problem affecting 1 in 27 women (Azizah et al., 2019). This reflects the enormity of breast cancer incidence, its impact and the need for effective prevention and treatment methods.

The mtDNA somatic mutations and their association with breast cancer have been studied extensively in the Western population and proven related but unexplored within the Malaysian population. Investigating somatic mutations and other alterations in the entire mtDNA involving Malaysian breast cancer patients may help to improve the knowledge gaps. Besides, breast cancer is highly diverse in its pathological features, with undetermined prognosis. Investigating other roles of mitochondria as the hub of oxidative stress and regulation of cell deaths in cancer through immunohistochemical marker analysis may pave the way towards better prognosis and therapeutic options.

1.4 Objective

1.4.1 General Objective

To investigate the associations between mtDNA mutations, oxidative stress, apoptosis, mitophagy and autophagy present in the Malaysian human breast cancer tissues.

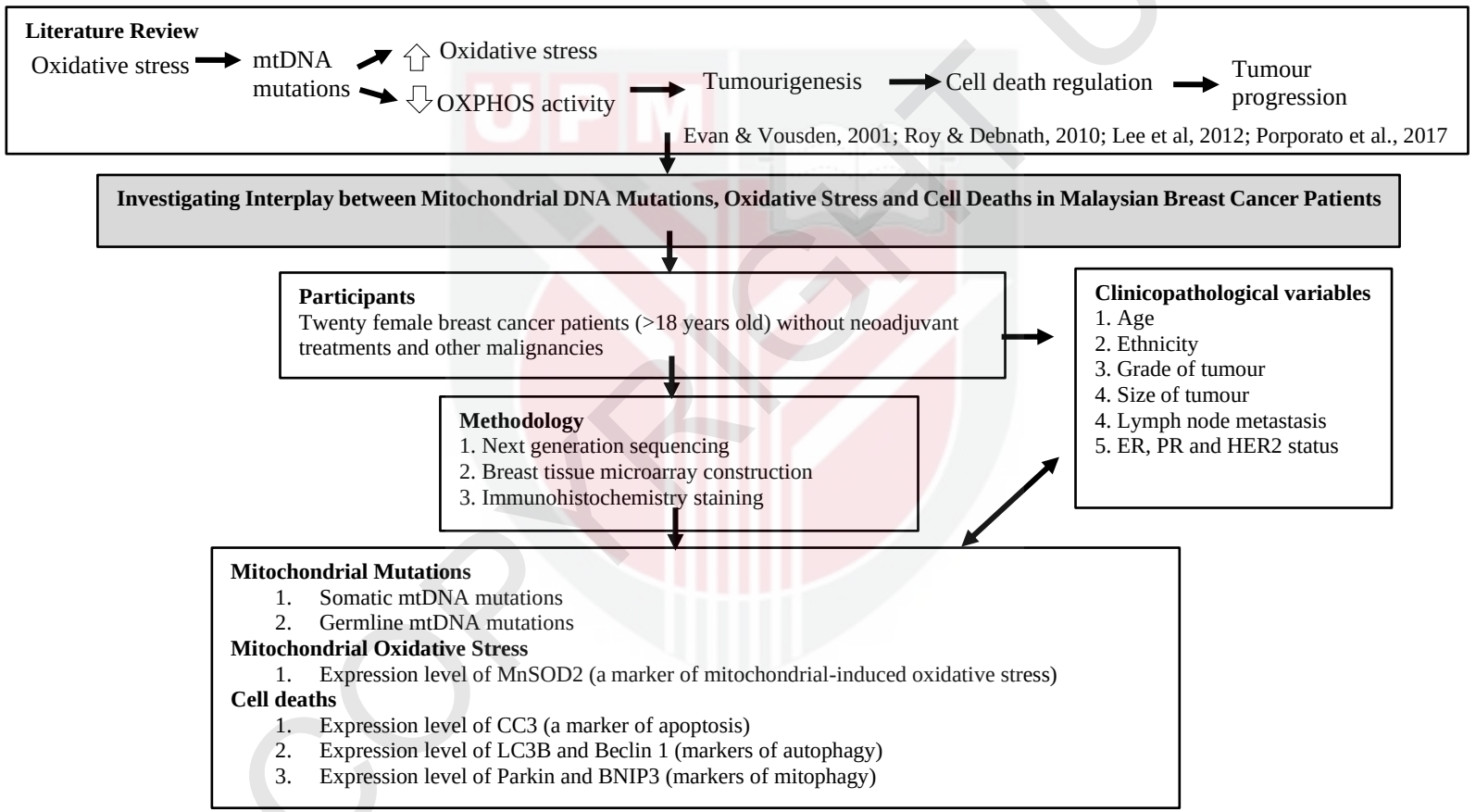
1.4.2 Specific Objective

1. To determine the type of mtDNA mutations present in Malaysian human breast cancer tissues and their association with the clinical pathophysiological parameters of the respective breast cancer patients.
2. To determine the levels of protein expression of MnSOD2, CC3, BNIP3, Parkin, LC3B and Beclin-1 in breast cancer and their association with clinical pathophysiological parameters of breast cancer patients.
3. To investigate the association between mtDNA mutations and the levels of protein expression of MnSOD2, CC3, BNIP3, Parkin, LC3B and Beclin-1 in breast cancer patients.

1.5 Hypothesis

Breast tumours and matched normal tissues are expected to have mutations in the mtDNA, either germline mutations and/or somatic mutations. The levels of expressions of MnSOD2, CC3, Beclin-1, LC3B, BNIP3 and Parkin are expected to be different between the normal tissues and breast tumour tissues. Mutations in the mtDNA may or may not be related to the expressions of MnSOD2, CC3, Beclin-1, LC3B, BNIP3 and Parkin in breast cancer. Mutations in mtDNA and levels of expressions of MnSOD2, CC3, Beclin-1, LC3B, BNIP3 and Parkin are expected to be different by increasing age groups, ethnicity, grades of tumours, stages of tumours and hormone receptors.

CONCEPTUAL FRAMEWORK



REFERENCES

Abubakar, M., Howat, W. J., Daley, F., Zabaglo, L., McDuffus, L. A., Blows, F., ... & Brenner, H. (2016). High-throughput automated scoring of Ki67 in breast cancer tissue microarrays from the Breast Cancer Association Consortium. *The Journal of Pathology: Clinical Research*, *2*(3), 138-153

Adey, A., Morrison, H. G., Xun, X., Kitzman, J. O., Turner, E. H., Stackhouse, B., ... & Shendure, J. (2010). Rapid, low-input, low-bias construction of shotgun fragment libraries by high-density in vitro transposition. *Genome biology*, *11*(12), R119.

Akhmedov, A. T., Rybin, V., & Marín-García, J. (2015). Mitochondrial oxidative metabolism and uncoupling proteins in the failing heart. *Heart failure reviews*, *20*(2), 227-249.

Alexeyev, M., Shokolenko, I., Wilson, G., & LeDoux, S. (2013). The maintenance of mitochondrial DNA integrity—critical analysis and update. Cold Spring Harbor perspectives in biology, 5(5), a012641.

Alhomidi, M. A., Vedicherla, B., Movva, S., Rao, P. K., Ahuja, Y. R., & Hasan, Q. (2013). Mitochondrial D310 instability in Asian Indian breast cancer patients. *Tumor Biology*, *34*(4), 2427-2432.

Alkushi, A. (2009). Validation of tissue microarray biomarker expression of breast carcinomas in Saudi women. *Hematology/oncology and stem cell therapy*, *2*(3), 394-398.

Allen, J. F. (2015). Why chloroplasts and mitochondria retain their own genomes and genetic systems: colocation for redox regulation of gene expression. *Proceedings of the National Academy of Sciences*, *112*(33), 10231-10238.

Allison, K. H. (2012). Molecular pathology of breast cancer: what a pathologist needs to know. *American journal of clinical pathology*, *138*(6), 770-780.

Alphelys. (2011). Tissue Arrayer Minicore ® 3. Retrieved Jun 21, 2019, from http://www.alphelys.com/alph01/prod/us/minicore/minicore.php

Andrews, R. M., Kubacka, I., Chinnery, P. F., Lightowlers, R. N., Turnbull, D. M., & Howell, N. (1999). Reanalysis and revision of the Cambridge reference sefence for human mitochondrial DNA. *Nature genetics*, *23*(2), 147-147.

Anita, B., Chan, C. A., Eznal, I. M. M., Harissa, H. H., Jennifer, L. S. M., Kenny, D. C. C., Mohd Aminuddin, M. Y., Nor Safariny. A., Noranizah, W., Noor Harzana, H., Nugnelis, R., Raflis, R.A., Rofiah, A., Siti, F. A. M. Z., Shahrun, N. A. S., Sofiah, Z. A., Winnie, O. P., Zahurin, I. (2019). Management of Breast Cancer (Third Edition). Putrajaya: Ministry of Health Malaysia, 3-5.

Archana, M., Yogesh, T. L., & Kumaraswamy, K. L. (2013). Various methods available for detection of apoptotic cells-A review. *Indian journal of cancer*, *50*(3), 274.

Arezi, P., & Rezvani, Z. (2018). The variation of mitochondrial NADH dehydrogenase subunit 4 (mtND4) and molecular dynamics simulation of SNPs among Iranian women with breast cancer. *Journal of Molecular Graphics and Modelling*, *85*, 242-249.

Asin-Cayuela, J., & Gustafsson, C. M. (2007). Mitochondrial transcription and its regulation in mammalian cells. *Trends in biochemical sciences*, *32*(3), 111-117.

Autenshlyus, A. I., Arkhipov, S. A., Kunts, T. A., Marinkin, I. O., Mikhailova, E. S., Karpukhina, X. V., & Varaksin, N. A. (2017). Cytokine profiles of tumor supernatants in invasive ductal cancer and fibroadenoma of the breast and its relationship with VEGF-A expression in the tumors. *International journal of immunopathology and pharmacology*, *30*(1), 83-88.

Avagliano, A., Ruocco, M. R., Aliotta, F., Belviso, I., Accurso, A., Masone, S., ... & Arcucci, A. (2019). Mitochondrial flexibility of breast cancers: a growth advantage and a therapeutic opportunity. Cells, 8(5), 401.

Azizah AM., Hashimah B., Nirmal K., Siti Zubaidah AR., Puteri NA., Nabihah A., Sukumaran R., Balqis B., Nadia SMR., Sharifah SSS., Rahayu O., Nur Alham O., Azlina AA. (2019). Malaysia National Cancer Registry Report (MNCR) 2012-2016. Putrajaya: Ministry of Health Malaysia, 31

Bai RK, Leal SM, Covarrubias D, Liu A and Wong LJ: Mitochondrial genetic background modifies breast cancer risk. Cancer Res. 67:4687–4894. 2007.

Baharudin, N. T., Jaafar, H., & Zainuddin, Z. (2012). Association of mitochondrial DNA 10398 polymorphism in invasive breast cancer in malay population of peninsular malaysia. *The Malaysian journal of medical sciences: MJMS*, *19*(1), 36.

Becuwe, P., Ennen, M., Klotz, R., Barbieux, C., & Grandemange, S. (2014). Manganese superoxide dismutase in breast cancer: from molecular mechanisms of gene regulation to biological and clinical significance. *Free Radical Biology and Medicine*, *77*, 139-151.

Bento, C. F., Renna, M., Ghislat, G., Puri, C., Ashkenazi, A., Vicinanza, M., ... & Rubinsztein, D. C. (2016). Mammalian autophagy: how does it work?. *Annual review of biochemistry*, *85*, 685-713.

Bernardini, J. P., Lazarou, M., & Dewson, G. (2017). Parkin and mitophagy in cancer. *Oncogene*, *36*(10), 1315-1327.

Bogenhagen, D. F. (2012). Mitochondrial DNA nucleoid structure. *Biochimica et Biophysica Acta (BBA)-Gene Regulatory Mechanisms*, *1819*(9-10), 914-920.

Borrelli, A., Schiattarella, A., Bonelli, P., Tuccillo, F. M., Buonaguro, F. M., & Mancini, A. (2014). The functional role of MnSOD as a biomarker of human diseases and therapeutic potential of a new isoform of a human recombinant MnSOD. *BioMed research international*, *2014*.

Bray, F., Ferlay, J., Soerjomataram, I., Siegel, R. L., Torre, L. A., & Jemal, A. (2018). Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA: a cancer journal for clinicians*, *68*(6), 394-424.

Briscoe, A. G., Hopkins, K. P., & Waeschenbach, A. (2016). High-throughput sequencing of complete mitochondrial genomes. In *Marine genomics* (pp. 45-64). Humana Press, New York, NY.

Bronner, I. F., Quail, M. A., Turner, D. J., & Swerdlow, H. (2013). Improved protocols for illumina sequencing. *Current protocols in human genetics*, *79*(1), 18-2.

Cai, F. F., Kohler, C., Zhang, B., Chen, W. J., Barekati, Z., Garritsen, H. S., ... & Zhong, X. Y. (2011). Mutations of mitochondrial DNA as potential biomarkers in breast cancer. *Anticancer research*, *31*(12), 4267-4271.

Calabrese, C., Simone, D., Diroma, M. A., Santorsola, M., Gutta, C., Gasparre, G., ... & Attimonelli, M. (2014). MToolBox: a highly automated pipeline for heteroplasmy annotation and prioritization analysis of human mitochondrial variants in high-throughput sequencing. *Bioinformatics*, *30*(21), 3115-3117.

Candas, D., & Li, J. J. (2014). MnSOD in oxidative stress response-potential regulation via mitochondrial protein influx. *Antioxidants & redox signaling*, *20*(10), 1599-1617.

Cardano, M., Diaferia, G. R., Falavigna, M., Spinelli, C. C., Sessa, F., DeBlasio, P., & Biunno, I. (2013). Cell and tissue microarray technologies for protein and nucleic acid expression profiling. Journal of Histochemistry & Cytochemistry, 61(2), 116-124.

Caruccio, N. (2011). Preparation of next-generation sequencing libraries using Nextera™ technology: simultaneous DNA fragmentation and adaptor tagging by in vitro transposition. In *High-Throughput Next Generation Sequencing* (pp. 241-255). Humana Press, Totowa, NJ.

Castellana, S., Rónai, J., & Mazza, T. (2015). MitImpact: an exhaustive collection of pre-computed pathogenicity predictions of human mitochondrial non-synonymous variants. *Human mutation*, *36*(2), E2413-E2422.

Chavan, S. S., Ravindra, S., & Prasad, M. S. N. (2017). Breast biomarkers-comparison on whole section and tissue microarray section. *Journal of Clinical and Diagnostic Research: JCDR*, *11*(3), EC40.

Chang, L., Weiner, L. S., Hartman, S. J., Horvath, S., Jeste, D., Mischel, P. S., & Kado, D. M. (2019). Breast cancer treatment and its effects on aging. Journal of geriatric oncology, 10(2), 346-355.

Chen, D. L., Engle, J. T., Griffin, E. A., Miller, J. P., Chu, W., Zhou, D., & Mach, R. H. (2015). Imaging caspase-3 activation as a marker of apoptosis-targeted treatment response in cancer. *Molecular Imaging and Biology*, *17*(3), 384-393.

Chen, X., Cho, D. B., & Yang, P. C. (2010). Double staining immunohistochemistry. *North American journal of medical sciences*, *2*(5), 241.

Chinnery, P. F., & Hudson, G. (2013). Mitochondrial genetics. *British medical bulletin*, *106*(1), 135-159.

Choi, J., Jung, W., & Koo, J. S. (2013). Expression of autophagy-related markers beclin-1, light chain 3A, light chain 3B and p62 according to the molecular subtype of breast cancer. *Histopathology*, *62*(2), 275-286.

Chourasia, A. H., Boland, M. L., & Macleod, K. F. (2015). Mitophagy and cancer. *Cancer & metabolism*, *3*(1), 1-11.

Chourasia, A. H., Tracy, K., Frankenberger, C., Boland, M. L., Sharifi, M. N., Drake, L. E., ... & Macleod, K. F. (2015). Mitophagy defects arising from BNip3 loss promote mammary tumor progression to metastasis. *EMBO reports*, *16*(9), 1145-1163.

Chuang, T. C., Liu, J. Y., Lin, C. T., Tang, Y. T., Yeh, M. H., Chang, S. C., ... & Kao, M. C. (2007). Human manganese superoxide dismutase suppresses HER2/neu-mediated breast cancer malignancy. *FEBS letters*, *581*(23), 4443-4449.

Church, S. L., Grant, J. W., Meese, E. U., & Trent, J. M. (1992). Sublocalization of the gene encoding manganese superoxide dismutase (MnSOD/SOD2) to 6q25 by fluorescence in situ hybridization and somatic cell hybrid mapping.

Clark, D.P., Pazdernik, N.J. (2013) Chapter e22- Mobile DNA. Molecular Biology (Second Edition)

CliniSciences (2020). Immunohistochemistry protocol- Avidin/Biotin Method (ABC). Retrieved August 31, 2020 from https://www.clinisciences.com/en/read/immunohistochemistry-immunofluorescence-659/immunohistochemistry-protocol-avidin-2216.html

Cole, A. J., Dwight, T., Gill, A. J., Dickson, K. A., Zhu, Y., Clarkson, A., ... & Marsh, D. J. (2016). Assessing mutant p53 in primary high-grade serous ovarian cancer using immunohistochemistry and massively parallel sequencing. Scientific reports, 6(1), 1-12.

Czarnecka, A. M., Krawczyk, T., Zdrożny, M., Lubiński, J., Arnold, R. S., Kukwa, W., ... & Petros, J. A. (2010). Mitochondrial NADH-dehydrogenase subunit 3 (ND3) polymorphism (A10398G) and sporadic breast cancer in Poland. *Breast cancer research and treatment*, *121*(2), 511-518.

De Matos, L. L., Trufelli, D. C., De Matos, M. G. L., & da Silva Pinhal, M. A. (2010). Immunohistochemistry as an important tool in biomarkers detection and clinical practice. *Biomarker insights*, *5*, BMI-S2185.

De Paepe, B. (2012). Mitochondrial markers for cancer: relevance to diagnosis, therapy, and prognosis and general understanding of malignant disease mechanisms. *International Scholarly Research Notices*, *2012*.

De Vitto, H., Mendonça, B. S., Elseth, K. M., Onul, A., Xue, J., Vesper, B. J., ... & Radosevich, J. A. (2013). Part III. Molecular changes induced by high nitric oxide adaptation in human breast cancer cell line BT-20 (BT-20-HNO): a switch from aerobic to anaerobic metabolism. *Tumor Biology*, *34*(1), 403-413.

Department of Statistics Malaysia. (2017, October 31). *Statistics on Causes of Death, Malaysia, 2017* [Press release]. Retrieved April 10, 2019 from https://www.dosm.gov.my

DiMauro, S., & Schon, E. A. (2001). Mitochondrial DNA mutations in human disease. *American journal of medical genetics*, *106*(1), 18-26.

Dong, M., Wan, X. B., Yuan, Z. Y., Wei, L., Fan, X. J., Wang, T. T., ... & Lin, Q. (2013). Low expression of Beclin 1 and elevated expression of HIF-1α refine distant metastasis risk and predict poor prognosis of ER-positive, HER2-negative breast cancer. *Medical oncology*, *30*(1), 355.

Drake, L. E., Springer, M. Z., Poole, L. P., Kim, C. J., & Macleod, K. F. (2017, December). Expanding perspectives on the significance of mitophagy in cancer. In *Seminars in cancer biology* (Vol. 47, pp. 110-124). Academic Press.

Dzulkifli, F. A., Mashor, M. Y., & Jaafar, H. (2019). An overview of recent counting methods for Ki67 IHC staining. *Journal of Biomedical and Clinical Sciences (JBCS)*, *3*(2), 10-17.

Ebner, S., Lang, R., Mueller, E. E., Eder, W., Oeller, M., Moser, A., ... & Kofler, B. (2011). Mitochondrial haplogroups, control region polymorphisms and malignant melanoma: a study in middle European Caucasians. *PLoS One*, *6*(12), e27192.

Evan, G. I., & Vousden, K. H. (2001). Proliferation, cell cycle and apoptosis in cancer. *Nature*, *411*(6835), 342-348.

Favaloro, B., Allocati, N., Graziano, V., Di Ilio, C., & De Laurenzi, V. (2012). Role of apoptosis in disease. *Aging (Albany NY)*, *4*(5), 330.

Fei, B., Ji, F., Chen, X., Liu, Z., Li, S., Mo, Z., & Fang, X. (2016). Expression and clinical significance of Beclin-1 in gastric cancer tissues of various clinical stages. *Oncology letters*, *11*(3), 2271-2277.

Fendt, L., Niederstätter, H., Huber, G., Zelger, B., Dünser, M., Seifarth, C., ... & Parson, W. (2011). Accumulation of mutations over the entire mitochondrial genome of breast cancer cells obtained by tissue microdissection. *Breast cancer research and treatment*, *128*(2), 327-336.

Feng, Y., Spezia, M., Huang, S., Yuan, C., Zeng, Z., Zhang, L., ... & Liu, B. (2018). Breast cancer development and progression: Risk factors, cancer stem cells, signaling pathways, genomics, and molecular pathogenesis. *Genes & diseases*, *5*(2), 77.

Fitzmaurice, C., Allen, C., Barber, R. M., Barregard, L., Bhutta, Z. A., Brenner, H., ... & Fleming, T. (2017). Global, regional, and national cancer incidence, mortality, years of life lost, years lived with disability, and disability-adjusted life-years for 32 cancer groups, 1990 to 2015: a systematic analysis for the global burden of disease study. *JAMA oncology*, *3*(4), 524-548.

Fu, A., Ma, S., Wei, N., Tan, B. X. X., Tan, E. Y., & Luo, K. Q. (2016). High expression of MnSOD promotes survival of circulating breast cancer cells and increases their resistance to doxorubicin. *Oncotarget*, *7*(31), 50239.

Geurts-Giele, W. R., Gathier, G. H., Atmodimedjo, P. N., Dubbink, H. J., & Dinjens, W. N. (2015). Mitochondrial D310 mutation as clonal marker for solid tumors. *Virchows Archiv*, *467*(5), 595-602.

Ghaffarpour, M., Mahdian, R., Fereidooni, F., Kamalidehghan, B., Moazami, N., & Houshmand, M. (2014). The mitochondrial ATPase6 gene is more susceptible to mutation than the ATPase8 gene in breast cancer patients. *Cancer cell international*, *14*(1), 21.

Ghatak, S., Lallawmzuali, D., Sapkota, R., Pautu, J. L., Muthukumaran, R. B., & Kumar, N. S. (2014). Mitochondrial D-loop and Cytochrome Oxidase C subunit I polymorphisms among the breast cancer patients of Mizoram, Northeast India. *Current genetics*, *60*(3), 201-212.

Glenn, T. C. (2011). Field guide to next-generation DNA sequencers. *Molecular ecology resources*, *11*(5), 759-769.

Glick, D., Barth, S., & Macleod, K. F. (2010). Autophagy: cellular and molecular mechanisms. *The Journal of pathology*, *221*(1), 3-12.

Gong, C., Bauvy, C., Tonelli, G., Yue, W., Delomenie, C., Nicolas, V., ... & Delbos, L. (2013). Beclin 1 and autophagy are required for the tumorigenicity of breast cancer stem-like/progenitor cells. *Oncogene*, *32*(18), 2261.

Grzybowska-Szatkowska, L., & Slaska, B. (2012). Polymorphisms in genes encoding mt-tRNA in female breast cancer in Poland. *Mitochondrial DNA*, *23*(2), 106-111.

Grzybowska-Szatkowska, L., Ślaska, B., Rzymowska, J., Brzozowska, A., & Floriańczyk, B. (2014). Novel mitochondrial mutations in the ATP6 and ATP8 genes in patients with breast cancer. *Molecular medicine reports*, *10*(4), 1772-1778.

Guo, Y., Li, J., Li, C. I., Shyr, Y., & Samuels, D. C. (2013). MitoSeek: extracting mitochondria information and performing high-throughput mitochondria sequencing analysis. *Bioinformatics*, *29*(9), 1210-1211.

Guo, W., Yang, D., Xu, H., Zhang, Y., Huang, J., Yang, Z., ... & Huang, Z. (2013). Mutations in the D-loop region and increased copy number of mitochondrial DNA in human laryngeal squamous cell carcinoma. Molecular biology reports, 40(1), 13-20.

Gupta, A., Anjomani-Virmouni, S., Koundouros, N., Dimitriadi, M., Choo-Wing, R., Valle, A., ... & Asara, J. M. (2017). PARK2 depletion connects energy and oxidative stress to PI3K/Akt activation via PTEN S-nitrosylation. *Molecular cell*, *65*(6), 999-1013.

Hahn, A., & Zuryn, S. (2019). The cellular mitochondrial genome landscape in disease. *Trends in Cell Biology*, *29*(3), 227-240.

Hart, P. C., Mao, M., De Abreu, A. L. P., Ansenberger-Fricano, K., Ekoue, D. N., Ganini, D., ... & Santos, J. H. (2015). MnSOD upregulation sustains the Warburg effect via mitochondrial ROS and AMPK-dependent signalling in cancer. *Nature communications*, *6*(1), 1-14.

Hassan, M., Watari, H., AbuAlmaaty, A., Ohba, Y., & Sakuragi, N. (2014). Apoptosis and molecular targeting therapy in cancer. *BioMed research international*, *2014*.

He, Y., Wu, J., Dressman, D. C., Iacobuzio-Donahue, C., Markowitz, S. D., Velculescu, V. E., ... & Papadopoulos, N. (2010). Heteroplasmic mitochondrial DNA mutations in normal and tumour cells. *Nature*, *464*(7288), 610.

He, Y., Zhao, X., Subahan, N. R., Fan, L., Gao, J., & Chen, H. (2014). The prognostic value of autophagy-related markers beclin-1 and microtubule-associated protein light chain 3B in cancers: a systematic review and meta-analysis. *Tumor Biology*, *35*(8), 7317-7326.

Everts, S. (2010). *Triggering The Kiss of Death. Small molecule spurs protein executioner into action*. Chemical & Engineering News. Retrieved Sept 23, 2020 from http://pubsapp.acs.org/cen/news/84/i38/8438molecule.html?

Hahn, A., & Zuryn, S. (2019). Mitochondrial genome (mtDNA) mutations that generate reactive oxygen species. Antioxidants, 8(9), 392.

Hertweck, K. L., & Dasgupta, S. (2017). The landscape of mtDNA modifications in cancer: a tale of two cities. *Frontiers in oncology*, *7*, 262.

Howison, M., Coetzer, M., & Kantor, R. (2019). Measurement error and variant-calling in deep Illumina sequencing of HIV. Bioinformatics, 35(12), 2029-2035.

Huang, Q., Li, F., Liu, X., Li, W., Shi, W., Liu, F. F., ... & Zhou, L. (2011). Caspase 3–mediated stimulation of tumor cell repopulation during cancer radiotherapy. *Nature medicine*, *17*(7), 860-866.

Hwang, B., Lee, J. H., & Bang, D. (2018). Single-cell RNA sequencing technologies and bioinformatics pipelines. *Experimental & molecular medicine*, *50*(8), 1-14.

Ibrahim, N. I., Dahlui, M., Aina, E. N., & Al-Sadat, N. (2012). Who are the breast cancer survivors in Malaysia. *Asian Pac J Cancer Prev*, *13*(5), 2213-2218.

Illumina, Inc. (2015). Calculating Percent Passing Filter for Patterned and Non-Patterned Flow Cells: A comparison of methods for calculating percent passing filter on Illumina flow cells. Retrieved Feb 20, 2021 https://www.illumina.com/content/dam/illumina-marketing/documents/products/technotes/hiseq-x-percent-pf-technical-note-770-2014-043.pdf

Illumina (2016). Miseq System Denature and Dilute Libraries Guide. Retrieved April 10, 2017 from https://support.illumina.com/downloads/prepare_libraries_for_sequencing_miseq_15039740.html

Illumina (2017). Nextera XT DNA Library Prep Kit Reference Guide. Retrieved April 10, 2017 from https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera-xt/nextera-xt-library-prep-reference-guide-15031942-03.pdf

Illumina (2018). Miseq System Guide. Retrieved Jun 10, 2019 from https://support.illumina.com/content/dam/illumina-support/documents/documentation/system_documentation/miseq/miseq-system-guide-for-miseq-reporter

Illumina (2020). Specifications for the Miseq System. Retrieved August 31, 2020 from https://www.illumina.com/systems/sequencing platforms/miseq/specifications.html

Imanishi, H., Hattori, K., Wada, R., Ishikawa, K., Fukuda, S., Takenaga, K., ... & Hayashi, J. I. (2011). Mitochondrial DNA mutations regulate metastasis of human breast cancer cells. *PloS one*, *6*(8), e23401.

Iommarini, L., Ghelli, A., Gasparre, G., & Porcelli, A. M. (2017). Mitochondrial metabolism and energy sensing in tumor progression. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1858*(8), 582-590.

Iommarini, L., Kurelac, I., Capristo, M., Calvaruso, M. A., Giorgio, V., Bergamini, C., ... & Fato, R. (2013). Different mtDNA mutations modify tumor progression in dependence of the degree of respiratory complex I impairment. *Human molecular genetics*, *23*(6), 1453-1466.

Ishikawa, K., Takenaga, K., Akimoto, M., Koshikawa, N., Yamaguchi, A., Imanishi, H., ... & Hayashi, J. I. (2008). ROS-generating mitochondrial DNA mutations can regulate tumor cell metastasis. Science, 320(5876), 661-664.

Ivell, R., Teerds, K., & Hoffman, G. E. (2014). Proper application of antibodies for immunohistochemical detection: antibody crimes and how to prevent them. *Endocrinology*, *155*(3), 676-687.

Jawhar, N. M. (2009). Tissue microarray: a rapidly evolving diagnostic and research tool. *Annals of Saudi medicine*, *29*(2), 123.

Jha, K., Shukla, M., Kumar, M., Shukla, V. K., & Pandey, M. (2017). Expression of Caspase 3 and Inhibition of Apoptosis Lowers Survival in Breast Cancer. *World Journal of Surgical Medical and Radiation Oncology*, *6*(3).

Jiang, P., & Mizushima, N. (2014). Autophagy and human diseases. *Cell research*, *24*(1), 69.

Jiménez-Morales, I., Cavaliere, S., Jones, D., & Rozière, J. (2018). Strong metal–support interaction improves activity and stability of Pt electrocatalysts on doped metal oxides. *Physical Chemistry Chemical Physics*, *20*(13), 8765-8772.

Jones, S., Anagnostou, V., Lytle, K., Parpart-Li, S., Nesselbush, M., Riley, D. R., ... & Galens, K. G. (2015). Personalized genomic analyses for cancer mutation discovery and interpretation. *Science translational medicine*, *7*(283), 283ra53-283ra53.

Ju, Y. S., Alexandrov, L. B., Gerstung, M., Martincorena, I., Nik-Zainal, S., Ramakrishna, M., ... & Bolli, N. (2014). Origins and functional consequences of somatic mitochondrial DNA mutations in human cancer. *Elife*, *3*, e02935.

Kamarajugadda, S., Cai, Q., Chen, H., Nayak, S., Zhu, J., He, M., ... & Tan, M. (2013). Manganese superoxide dismutase promotes anoikis resistance and tumor metastasis. *Cell death & disease*, *4*(2), e504.

Kane, L. A., Lazarou, M., Fogel, A. I., Li, Y., Yamano, K., Sarraf, S. A., ... & Youle, R. J. (2014). PINK1 phosphorylates ubiquitin to activate Parkin E3 ubiquitin ligase activity. *J Cell Biol*, *205*(2), 143-153.

Kang, E., Wang, X., Tippner-Hedges, R., Ma, H., Folmes, C. D., Gutierrez, N. M., ... & Koski, A. (2016). Age-related accumulation of somatic mitochondrial DNA mutations in adult-derived human iPSCs. *Cell stem cell*, *18*(5), 625-636.

Kauppila, J. H., & Stewart, J. B. (2015). Mitochondrial DNA: Radically free of free-radical driven mutations. *Biochimica et Biophysica Acta (BBA)-Bioenergetics*, *1847*(11), 1354-1361.

Khoury, T., Zirpoli, G., Cohen, S. M., Geradts, J., Omilian, A., Davis, W., ... & Palmer, J. R. (2017). Ki-67 expression in breast cancer tissue microarrays: assessing tumor heterogeneity, concordance with full section, and scoring methods. *American journal of clinical pathology*, *148*(2), 108-118.

Kia, A., Gloeckner, C., Osothprarop, T., Gormley, N., Bomati, E., Stephenson, M., ... & He, M. M. (2017). Improved genome sequencing using an engineered transposase. *BMC biotechnology*, *17*(1), 6.

Kim, A. (2014). Mitochondrial DNA somatic mutation in cancer. *Toxicological research*, *30*(4), 235-242.

Kim, J. Y., Jung, W. H., & Koo, J. S. (2014). Expression of autophagy-related proteins according to androgen receptor and HER-2 status in estrogen receptor-negative breast cancer. *PloS one*, *9*(8), e105666.

Kim, Y. S., Gupta Vallur, P., Phaëton, R., Mythreye, K., & Hempel, N. (2017). Insights into the Dichotomous Regulation of SOD2 in Cancer. *Antioxidants*, *6*(4), 86.

Kircher, M., Stenzel, U., & Kelso, J. (2009). Improved base calling for the Illumina Genome Analyzer using machine learning strategies. *Genome biology*, *10*(8), R83.

Kirches, E. (2017). MtDNA as a cancer marker: a finally closed chapter?. *Current genomics*, *18*(3), 255-267.

Kloss-Brandstätter, A., Schäfer, G., Erhart, G., Hüttenhofer, A., Coassin, S., Seifarth, C., ... & Kronenberg, F. (2010). Somatic mutations throughout the entire mitochondrial genome are associated with elevated PSA levels in prostate cancer patients. *The American Journal of Human Genetics*, *87*(6), 802-812.

Kloss-Brandstätter, A., Weissensteiner, H., Erhart, G., Schäfer, G., Forer, L., Schönherr, S., ... & Sottsas, I. (2015). Validation of next-generation sequencing of entire mitochondrial genomes and the diversity of mitochondrial DNA mutations in oral squamous cell carcinoma. *PLoS One*, *10*(8), e0135643.

Kodack, D. P., Farago, A. F., Dastur, A., Held, M. A., Dardaei, L., Friboulet, L., ... & Dicecca, R. (2017). Primary patient-derived cancer cells and their potential for personalized cancer patient care. *Cell reports*, *21*(11), 3298-3309.

Kondapalli, C., Kazlauskaite, A., Zhang, N., Woodroof, H. I., Campbell, D. G., Gourlay, R., ... & Knebel, A. (2012). PINK1 is activated by mitochondrial membrane potential depolarization and stimulates Parkin E3 ligase activity by phosphorylating Serine 65. *Open biology*, *2*(5), 120080.

Koop, E. A., van Laar, T., van Wichen, D. F., de Weger, R. A., van der Wall, E., & van Diest, P. J. (2009). Expression of BNIP3 in invasive breast cancer: correlations with the hypoxic response and clinicopathological features. *BMC cancer*, *9*(1), 1-8.

Kulikov, A. V., Luchkina, E. A., Gogvadze, V., & Zhivotovsky, B. (2017). Mitophagy: Link to cancer development and therapy. *Biochemical and biophysical research communications*, *482*(3), 432-439.

Kumar, A. P., Loo, S. Y., Shin, S. W., Tan, T. Z., Eng, C. B., Singh, R., ... & Park, J. I. (2014). Manganese superoxide dismutase is a promising target for enhancing chemosensitivity of basal-like breast carcinoma. *Antioxidants & redox signaling*, *20*(15), 2326-2346.

Kuo, S. J., Chen, M., Ma, G. C., Chen, S. T., Chang, S. P., Lin, W. Y., ... & Liu, C. S. (2010). Number of somatic mutations in the mitochondrial D-loop region indicates poor prognosis in breast cancer, independent of TP53 mutation. *Cancer genetics and cytogenetics*, *201*(2), 94-101.

Ladoire, S., Chaba, K., Martins, I., Sukkurwala, A. Q., Adjemian, S., Michaud, M., ... & Rosenfeldt, M. (2012). Immunohistochemical detection of cytoplasmic LC3 puncta in human cancer specimens. *Autophagy*, *8*(8), 1175-1184.

Larman, T. C., DePalma, S. R., Hadjipanayis, A. G., Protopopov, A., Zhang, J., Gabriel, S. B., ... & Cancer Genome Atlas Research Network. (2012). Spectrum of somatic mitochondrial mutations in five cancers. *Proceedings of the National Academy of Sciences*, *109*(35), 14087-14091.

Laronga, C., Chagpar, A. B., & Vora, S. R. (2016). Patient education: breast cancer guide to diagnosis and treatment (beyond the basics). *UpToDate. UpToDate, Waltham. https://www. uptodate. com/contents/breast-cancer-guide-to-diagnosis-and-treatment-beyond-the-basics. Accessed*, *7*

Lazova, R., Camp, R. L., Klump, V., Siddiqui, S. F., Amaravadi, R. K., & Pawelek, J. M. (2012). Punctate LC3B expression is a common feature of solid tumors and associated with proliferation, metastasis, and poor outcome. *Clinical cancer research*, *18*(2), 370-379.

Lee, H. C., Chang, C. M., & Chi, C. W. (2010). Somatic mutations of mitochondrial DNA in aging and cancer progression. *Ageing research reviews*, *9*, S47-S58.

Lee, J., Giordano, S., & Zhang, J. (2012). Autophagy, mitochondria and oxidative stress: cross-talk and redox signalling. *Biochemical Journal*, *441*(2), 523-540.

Lemarie, A., & Grimm, S. (2011). Mitochondrial respiratory chain complexes: apoptosis sensors mutated in cancer?. *Oncogene*, *30*(38), 3985.

Levine, A. J., & Puzio-Kuter, A. M. (2010). The control of the metabolic switch in cancers by oncogenes and tumor suppressor genes. *Science*, *330*(6009), 1340-1344.

Li, B., Krishnan, V. G., Mort, M. E., Xin, F., Kamati, K. K., Cooper, D. N., ... & Radivojac, P. (2009). Automated inference of molecular mechanisms of disease from amino acid substitutions. *Bioinformatics*, *25*(21), 2744-2750.

Li, L., Chen, L., Li, J., Zhang, W., Liao, Y., Chen, J., & Sun, Z. (2016). Correlational study on mitochondrial DNA mutations as potential risk factors in breast cancer. *Oncotarget*, *7*(21), 31270.

Li, Y., Zhang, X., Yang, J., Zhang, Y., Zhu, D., Zhang, L., ... & Zhou, J. (2017). Methylation of BNIP3 in pancreatic cancer inhibits the induction of mitochondrial-mediated tumor cell apoptosis. *Oncotarget*, *8*(38), 63208.

Li, Z., Chen, B., Wu, Y., Jin, F., Xia, Y., & Liu, X. (2010). Genetic and epigenetic silencing of the beclin 1 gene in sporadic breast tumors. *BMC cancer*, *10*(1), 98.

Liang, X. H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., & Levine, B. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature*, *402*(6762), 672.

Lim, G. C. C., Yahaya, H., & Lim, T. O. (2003). The first report of the national cancer registry cancer incidence in Malaysia. Kuala Lumpur: Ministry of Health, 57-58.

Lin, C. S., Chang, S. C., Ou, L. H., Chen, C. M., Hsieh, S. S. W., Chung, Y. P., ... & Wei, Y. H. (2015). Mitochondrial DNA alterations correlate with the pathological status and the immunological ER, PR, HER-2/neu, p53 and Ki-67 expression in breast invasive ductal carcinoma. *Oncology reports*, *33*(6), 2924-2934.

Lin, J., Jordi, C., Son, M., Van Phan, H., Drayman, N., Abasiyanik, M. F., ... & Tay, S. (2019). Ultra-sensitive digital quantification of proteins and mRNA in single cells. *Nature communications*, *10*(1), 1-10.

Liou, C. W., Lin, T. K., Chen, J. B., Tiao, M. M., Weng, S. W., Chen, S. D., ... & Wang, P. W. (2010). Association between a common mitochondrial DNA D-loop polycytosine variant and alteration of mitochondrial copy number in human peripheral blood cells. *Journal of medical genetics*, *47*(11), 723-728.

Liou, G. Y., & Storz, P. (2010). Reactive oxygen species in cancer. *Free radical research*, *44*(5), 479-496.

Liu, J., Zhang, C., Zhao, Y., Yue, X., Wu, H., Huang, S., ... & Gatza, M. L. (2017). Parkin targets HIF-1α for ubiquitination and degradation to inhibit breast tumor progression. *Nature communications*, *8*(1), 1823.

Liu, X., Jiang, S., Tian, X., & Jiang, Y. (2018). Expression of cleaved caspase-3 predicts good chemotherapy response but poor survival for patients with advanced primary triple-negative breast cancer. *International Journal of Clinical and Experimental Pathology*, *11*(9), 4363.

Lott, M. T., Leipzig, J. N., Derbeneva, O., Xie, H. M., Chalkia, D., Sarmady, M., ... & Wallace, D. C. (2013). mtDNA variation and analysis using MITOMAP and MITOMASTER. *Current protocols in bioinformatics*, *44*(1), 1-23.

Lv, M., Wang, C., Li, F., Peng, J., Wen, B., Gong, Q., ... & Tang, Y. (2017). Structural insights into the recognition of phosphorylated FUNDC1 by LC3B in mitophagy. *Protein & cell*, *8*(1), 25-38.

McElhoe, J. A., Holland, M. M., Makova, K. D., Su, M. S. W., Paul, I. M., Baker, C. H., ... & Young, B. (2014). Development and assessment of an optimized next-generation DNA sequencing approach for the mtgenome using the Illumina MiSeq. *Forensic Science International: Genetics*, *13*, 20-29.

McIlwain, D. R., Berger, T., & Mak, T. W. (2013). Caspase functions in cell death and disease. *Cold Spring Harbor perspectives in biology*, *5*(4), a008656.

McIlwain, D. R., Berger, T., & Mak, T. W. (2015). Caspase functions in cell death and disease. *Cold Spring Harbor Perspectives in Biology*, *7*(4).

McMahon, S., & LaFramboise, T. (2014). Mutational patterns in the breast cancer mitochondrial genome, with clinical correlates. *Carcinogenesis*, *35*(5), 1046-1054.

Meng, X. L., Meng, H., Zhang, W., Qin, Y. H., & Zhao, N. M. (2016). The role of mitochondrial tRNA variants in female breast cancer. *Mitochondrial DNA Part A*, *27*(5), 3199-3201.

Metzker, M. L. (2010). Sequencing technologies—the next generation. *Nature reviews genetics*, *11*(1), 31.

Michiorri, S., Gelmetti, V., Giarda, E., Lombardi, F., Romano, F., Marongiu, R., ... & Torosantucci, L. (2010). The Parkinson-associated protein PINK1 interacts with Beclin1 and promotes autophagy. Cell Death & Differentiation, 17(6), 962-974.

Ministry of Health Malaysia. (2010). *Management of Breast Cancer 2nd Edition*. Retrieved April 10, 2019 from http://www.moh.gov.my

Mizushima, N., Yoshimori, T., & Levine, B. (2010). Methods in mammalian autophagy research. *Cell*, *140*(3), 313-326.

Mohamed Yusoff, A. A., Mohd Nasir, K. N., Haris, K., Mohd Khair, S. Z. N., Abdul Ghani, A. R. I., Idris, Z., & Abdullah, J. M. (2017). Detection of somatic mutations in the mitochondrial DNA control region D-loop in brain tumors: The first report in Malaysian patients. *Oncology letters*, *14*(5), 5179-5188.

Moraes, C. T. (2001). What regulates mitochondrial DNA copy number in animal cells?. *TRENDS in Genetics*, *17*(4), 199-205.

Muzzey, D., Evans, E. A., & Lieber, C. (2015). Understanding the basics of NGS: from mechanism to variant calling. *Current genetic medicine reports*, *3*(4), 158-165.

Nagpal, M., Singh, S., Singh, P., Chauhan, P., & Zaidi, M. A. (2016). Tumor markers: A diagnostic tool. *National journal of maxillofacial surgery*, *7*(1), 17.

Nakazato, T., Ohta, T., & Bono, H. (2013). Experimental design-based functional mining and characterization of high-throughput sequencing data in the sequence read archive. PLoS One, 8(10), e77910.

National Cancer Institute, Ministry of Health Malaysia (2016). *Malaysian National Cancer Registry Report 2007–2011*. Retrieved April 10, 2019 from http://nci.moh.gov.my

Niu, Y., Lin, Z., Wan, A., Chen, H., Liang, H., Sun, L., ... & Wu, X. (2019). RNA N6-methyladenosine demethylase FTO promotes breast tumor progression through inhibiting BNIP3. *Molecular cancer*, *18*(1), 46.

Nushtaeva, A. A., Karpushina, A. A., Ermakov, M. S., Gulyaeva, L. F., Gerasimov, A. V., Sidorov, S. V., ... & Koval, O. A. (2019). Establishment of primary human breast cancer cell lines using "pulsed hypoxia" method and development of metastatic tumor model in immunodeficient mice. *Cancer Cell International*, *19*(1), 1-19.

Oberley, L. W. (2005). Mechanism of the tumor suppressive effect of MnSOD overexpression. *Biomedicine & Pharmacotherapy*, *59*(4), 143-148.

Okamoto, K., & Kondo-Okamoto, N. (2012). Mitochondria and autophagy: critical interplay between the two homeostats. *Biochimica et Biophysica Acta (BBA)-General Subjects*, *1820*(5), 595-600.

Osellame, L. D., Blacker, T. S., & Duchen, M. R. (2012). Cellular and molecular mechanisms of mitochondrial function. *Best practice & research Clinical endocrinology & metabolism*, *26*(6), 711-723.

Oyejide, L., Mendes, O. R., & Mikaelian, I. (2017). *Molecular Pathology. A Comprehensive Guide to Toxicology in Nonclinical Drug Development, 407–445.*

Ozpolat, B., & Benbrook, D. M. (2015). Targeting autophagy in cancer management–strategies and developments. *Cancer management and research*, *7*, 291.

Pagani, I. S., Kok, C. H., Saunders, V. A., Van der Hoek, M. B., Heatley, S. L., Schwarer, A. P., ... & Ross, D. M. (2017). A method for next-generation sequencing of paired diagnostic and remission samples to detect mitochondrial DNA mutations associated with Leukemia. *The Journal of Molecular Diagnostics*, *19*(5), 711-721.

Parker, R. L., Huntsman, D. G., Lesack, D. W., Cupples, J. B., Grant, D. R., Akbari, M., & Gilks, C. B. (2002). Assessment of interlaboratory variation in the immunohistochemical determination of estrogen receptor status using a breast cancer tissue microarray. *American journal of clinical pathology*, *117*(5), 723-728.

Pathy, N. B., Yip, C. H., Taib, N. A., Hartman, M., Saxena, N., Iau, P., ... & Verkooijen, H. M. (2011). Breast cancer in a multi-ethnic Asian setting: results from the Singapore–Malaysia hospital-based breast cancer registry. *The Breast*, *20*, S75-S80.

Pérez-Amado, C. J., Tovar, H., Gómez-Romero, L., Beltrán-Anaya, F. O., Bautista-Piña, V., Dominguez-Reyes, C., ... & Jiménez-Morales, S. (2020). Mitochondrial DNA Mutation Analysis in Breast Cancer: Shifting From Germline Heteroplasmy Toward Homoplasmy in Tumors. Frontiers in oncology, 10.

Petric, R. C., Pop, L. A., Jurj, A., Raduly, L., Dumitrascu, D., Dragos, N., & Neagoe, I. B. (2015). Next generation sequencing applications for breast cancer research. *Clujul medical*, 88(3), 278.

Pfeffer, C. M., & Singh, A. T. (2018). Apoptosis: a target for anticancer therapy. *International journal of molecular sciences*, *19*(2), 448.

Picca, A., Guerra, F., Calvani, R., Coelho-Junior, H. J., Bossola, M., Landi, F., ... & Marzetti, E. (2020). Generation and Release of Mitochondrial-Derived Vesicles in Health, Aging and Disease. *Journal of Clinical Medicine*, 9(5), 1440.

Porporato, P. E., Filigheddu, N., Bravo-San Pedro, J. M., Kroemer, G., & Galluzzi, L. (2018). Mitochondrial metabolism and cancer. *Cell research*, *28*(3), 265-280.

Provenzano, E., Ulaner, G. A., & Chin, S. F. (2018). Molecular classification of breast cancer. *PET clinics*, *13*(3), 325-338.

Pu, X., Storr, S. J., Zhang, Y., Rakha, E. A., Green, A. R., Ellis, I. O., & Martin, S. G. (2017). Caspase-3 and caspase-8 expression in breast cancer: caspase-3 is associated with survival. *Apoptosis*, *22*(3), 357-368.

Qiagen (2016). QIAamp® DNA Mini and Blood Mini Handbook. Retrieved Jan 10, 2017 from https://www.qiagen.com/en

Qu, X., Yu, J., Bhagat, G., Furuya, N., Hibshoosh, H., Troxel, A., ... & Cattoretti, G. (2003). Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. *The Journal of clinical investigation*, *112*(12), 1809-1820.

Rambold, A. S., & Lippincott-Schwartz, J. (2011). Mechanisms of mitochondria and autophagy crosstalk. *Cell cycle*, *10*(23), 4032-4038.

Rasheed, B. A., Stenzel, T. T., McLendon, R. E., Parsons, R., Friedman, A. H., Friedman, H. S., ... & Bigner, S. H. (1997). PTEN gene mutations are seen in high-grade but not in low-grade gliomas. Cancer research, 57(19), 4187-4190.

Robberson, D. L., Kasamatsu, H., & Vinograd, J. (1972). Replication of mitochondrial DNA. Circular replicative intermediates in mouse L cells. *Proceedings of the National Academy of Sciences of the United States of America*, *69*(3), 737.

Roger, A. J., Muñoz-Gómez, S. A., & Kamikawa, R. (2017). The origin and diversification of mitochondria. *Current Biology*, *27*(21), R1177-R1192.

Roy, S., & Debnath, J. (2010, December). Autophagy and tumorigenesis. In *Seminars in immunopathology* (Vol. 32, No. 4, pp. 383-396). Springer-Verlag.

Santorsola, M., Calabrese, C., Girolimetti, G., Diroma, M. A., Gasparre, G., & Attimonelli, M. (2016). A multi-parametric workflow for the prioritization of mitochondrial DNA variants of clinical interest. *Human genetics*, *135*(1), 121-136.

Sarmiento-Salinas, F. L., Delgado-Magallón, A., Montes-Alvarado, J. B., Ramírez-Ramírez, D., Flores-Alonso, J. C., Cortés-Hernández, P., ... & Millán-Pérez-Peña, L. (2019). Breast cancer subtypes present a differential production of reactive oxygen species (ROS) and Susceptibility to antioxidant treatment. *Frontiers in oncology*, *9*, 480.

Sazonova, S., Grube, M., Shvirksts, K., Galoburda, R., & Gramatina, I. (2019). FTIR spectroscopy studies of high pressure-induced changes in pork macromolecular structure. *Journal of Molecular Structure*, *1186*, 377-383.

Schieber, M., & Chandel, N. S. (2014). ROS function in redox signaling and oxidative stress. *Current biology*, *24*(10), R453-R462.

Shafik, Y. S., Rafik, H., & Helmy, M. (2018). Factors contributing to local recurrence after conservative breast surgery for early-stage breast cancer. *The Egyptian Journal of Surgery*, *37*(4), 429.

Shah, R., Rosso, K., & Nathanson, S. D. (2014). Pathogenesis, prevention, diagnosis and treatment of breast cancer. *World journal of clinical oncology*, *5*(3), 283.

Sheikh, M. K., Khan, F. A., Imran Abdul Khalid, I. K., & Kumar, G. (2009). Age specific histologic types of carcinoma breast in Malaysians. *J Coll Physicians Surg Pak*, *19*(3), 201-202.

Shen J, Wan J, Song R, Zhao H. Peripheral blood mitochondrial DNA copy number, length heteroplasmy and breast cancer risk: a replication study. Carcinogenesis. (2015) 36:1307–13. doi: 10.1093/carcin/bgv130

Shendure, J., & Aiden, E. L. (2012). The expanding scope of DNA sequencing. *Nature biotechnology*, *30*(11), 1084.

Sherry, S. T., Ward, M. H., Kholodov, M., Baker, J., Phan, L., Smigielski, E. M., & Sirotkin, K. (2001). dbSNP: the NCBI database of genetic variation. *Nucleic acids research*, *29*(1), 308-311.

Shidara, Y., Yamagata, K., Kanamori, T., Nakano, K., Kwong, J. Q., Manfredi, G., ... & Ohta, S. (2005). Positive contribution of pathogenic mutations in the mitochondrial genome to the promotion of cancer by prevention from apoptosis. *Cancer research*, *65*(5), 1655-1663.

Siegel, R. L., Miller, K. D., & Jemal, A. (2016). Cancer statistics, 2016. *CA: a cancer journal for clinicians*, *66*(1), 7-30.

Smith, P. M., Elson, J. L., Greaves, L. C., Wortmann, S. B., Rodenburg, R. J., Lightowlers, R. N., ... & Vila-Sanjurjo, A. (2013). The role of the mitochondrial ribosome in human disease: searching for mutations in 12S mitochondrial rRNA with high disruptive potential. *Human molecular genetics*, *23*(4), 949-967.

Snezhkina, A. V., Kudryavtseva, A. V., Kardymon, O. L., Savvateeva, M. V., Melnikova, N. V., Krasnov, G. S., & Dmitriev, A. A. (2019). ROS generation and antioxidant defense systems in normal and malignant cells. *Oxidative medicine and cellular longevity*, *2019*.

Soini, Y., Vakkala, M., Kahlos, K., Pääkkö, P., & Kinnula, V. (2001). MnSOD expression is less frequent in tumour cells of invasive breast carcinomas than in in situ carcinomas or non-neoplastic breast epithelial cells. *The Journal of pathology*, *195*(2), 156-162.

Soliman, N. A., & Yussif, S. M. (2016). Ki-67 as a prognostic marker according to breast cancer molecular subtype. *Cancer biology & medicine*, *13*(4), 496.

Spoelstra, N. S., Cittelly, D. M., Christenson, J. L., Gordon, M. A., Elias, A., Jedlicka, P., & Richer, J. K. (2016). Dicer expression in estrogen receptor–positive versus triple-negative breast cancer: an antibody comparison. *Human pathology*, *56*, 40-51.

Springer, M. Z., & Macleod, K. F. (2016). In brief: mitophagy: mechanisms and role in human disease. *The Journal of pathology*, *240*(3), 253-255.

Stawski, H. (2013). *Preparing whole genome human mitochondrial DNA libraries for next generation sequencing using Illumina Nextera XT*. Western Carolina University.

Stewart, J. B., Alaei-Mahabadi, B., Sabarinathan, R., Samuelsson, T., Gorodkin, J., Gustafsson, C. M., & Larsson, E. (2015). Simultaneous DNA and RNA mapping of somatic mitochondrial mutations across diverse human cancers. PLoS genetics, 11(6), e1005333.

Stoneking, M. (2000). Hypervariable sites in the mtDNA control region are mutational hotspots. *The American Journal of Human Genetics*, *67*(4), 1029-1032.

Stranneheim, H., & Lundeberg, J. (2012). Stepping stones in DNA sequencing. *Biotechnology journal*, *7*(9), 1063-1073.

Suen, D. F., Narendra, D. P., Tanaka, A., Manfredi, G., & Youle, R. J. (2010). Parkin overexpression selects against a deleterious mtDNA mutation in heteroplasmic cybrid cells. *Proceedings of the National Academy of Sciences*, *107*(26), 11835-11840.

Sylvester, P. W., & Tiwari, R. V. (2016). Role of Autophagy in Mediating the Anticancer Effects of Tocotrienols. *Autophagy in Current Trends in Cellular Physiology and Pathology*, 89.

Tan, D. J., Bai, R. K., & Wong, L. J. C. (2002). Comprehensive scanning of somatic mitochondrial DNA mutations in breast cancer. *Cancer research*, *62*(4), 972-976.

Taneja, N., Kukal, S., & Mani, S. (2015). Cytb: A hot spot for pathogenic mutations in mitochondrial genome of breast cancer and ovarian cancer patients. *International Journal of Pharmacy and Pharmaceutical Sciences*, *7*(9), 128-135.

Tang, S., Wang, J., Zhang, V. W., Li, F. Y., Landsverk, M., Cui, H., ... & Scaglia, F. (2013). Transition to next generation analysis of the whole mitochondrial genome: a summary of molecular defects. *Human mutation*, *34*(6), 882-893.

Tao, Z., Shi, A., Lu, C., Song, T., Zhang, Z., & Zhao, J. (2015). Breast cancer: epidemiology and etiology. *Cell biochemistry and biophysics*, *72*(2), 333-338.

Tay, S. P., Yeo, C. W., Chai, C., Chua, P. J., Tan, H. M., Ang, A. X., ... & Wong, S. H. (2010). Parkin enhances the expression of cyclin-dependent kinase 6 and negatively regulates the proliferation of breast cancer cells. *Journal of Biological Chemistry*, *285*(38), 29231-29238.

Tommasi, S., Favia, P., Weigl, S., Bianco, A., Pilato, B., Russo, L., ... & Petruzzella, V. (2014). Mitochondrial DNA variants and risk of familial breast cancer: an exploratory study. *International journal of oncology*, *44*(5), 1691-1698.

Turashvili, G., & Brogi, E. (2017). Tumor heterogeneity in breast cancer. *Frontiers in medicine*, *4*, 227.

Tvrdik, D., Staněk, L., Skalova, H., Dundr, P., Velenská, Z., & Povýšil, C. (2012). Comparison of the IHC, FISH, SISH and qPCR methods for the molecular diagnosis of breast cancer. *Molecular medicine reports*, *6*(2), 439-443.

Vakifahmetoglu-Norberg, H., Ouchida, A. T., & Norberg, E. (2017). The role of mitochondria in metabolism and cell death. *Biochemical and biophysical research communications*, *482*(3), 426-431.

Vartak, R., Deng, J., Fang, H., & Bai, Y. (2015). Redefining the roles of mitochondrial DNA-encoded subunits in respiratory Complex I assembly. *Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease*, *1852*(7), 1531-1539.

Vector Laboratories.(2019). Vectastain® Elite® ABC HRP Kit. Retrieved Jun 22, 2019, from https://vectorlabs.com/vectastain-elite-abc-kit-rabbit-igg.html

Vellarikkal, S. K., Dhiman, H., Joshi, K., Hasija, Y., Sivasubbu, S., & Scaria, V. (2015). mit-o-matic: A Comprehensive Computational Pipeline for Clinical Evaluation of Mitochondrial Variations from Next-Generation Sequencing Datasets. *Human mutation*, *36*(4), 419-424.

Venkitaraman, R. (2010). Lobular neoplasia of the breast. *The breast journal*, *16*(5), 519-528.

Vernucci, E., Tomino, C., Molinari, F., Limongi, D., Aventaggiato, M., Sansone, L., ... & Russo, M. A. (2019). Mitophagy and Oxidative Stress in Cancer and Aging: Focus on Sirtuins and Nanomaterials. *Oxidative Medicine and Cellular Longevity*, *2019*.

Wahabi, H. A., Esmaeil, S. A., Bahkali, K. H., Titi, M. A., Amer, Y. S., Fayed, A. A., ... & Car, L. T. (2019). Medical Doctors' Offline Computer-Assisted Digital Education: Systematic Review by the Digital Health Education Collaboration. *Journal of medical Internet research*, *21*(3), e12998.

Wallace, D. C. (2012). Mitochondria and cancer. *Nature Reviews Cancer*, *12*(10), 685-698.

Wang, J., Pan, X. L., Ding, L. J., Liu, D. Y., Lei, D. P., & Jin, T. (2013). Aberrant expression of Beclin-1 and LC3 correlates with poor prognosis of human hypopharyngeal squamous cell carcinoma. *PloS one*, *8*(7), e69038.

Wanger, A., Chavez, V., Huang, R., Wahed, A., Dasgupta, A., & Actor, J. K. (2017). *Microbiology and Molecular Diagnosis in Pathology: A Comprehensive Review for Board Preparation, Certification and Clinical Practice*. Elsevier.

Watts, L. T., Lloyd, R., Garling, R. J., & Duong, T. (2013). Stroke neuroprotection: targeting mitochondria. *Brain sciences*, *3*(2), 540-560.

Weigl, S., Paradiso, A., & Tommasi, S. (2013). Mitochondria and familial predisposition to breast cancer. Current genomics, 14(3), 195.

Weerts, M. J., Sleijfer, S., & Martens, J. W. (2019). The role of mitochondrial DNA in breast tumors. *Drug Discovery Today*, *24*(5), 1202-1208.

Weissensteiner, H., Forer, L., Fuchsberger, C., Schöpf, B., Kloss-Brandstätter, A., Specht, G., ... & Schönherr, S. (2016). mtDNA-Server: next-generation sequencing data analysis of human mitochondrial DNA in the cloud. *Nucleic acids research*, *44*(W1), W64-W69.

Wilczynski, S. P. (2009). *Molecular Biology. Modern Surgical Pathology, 85–120.*

Wilkins, H. M., Carl, S. M., & Swerdlow, R. H. (2014). Cytoplasmic hybrid (cybrid) cell lines as a practical model for mitochondriopathies. *Redox biology*, *2*, 619-631.

Won, K. Y., Kim, G. Y., Kim, Y. W., Song, J. Y., & Lim, S. J. (2010). Clinicopathologic correlation of beclin-1 and bcl-2 expression in human breast cancer. *Human pathology*, *41*(1), 107-112.

Wong, R. S. (2011). Apoptosis in cancer: from pathogenesis to treatment. *Journal of Experimental & Clinical Cancer Research*, *30*(1), 87.

Xu, H., Eirew, P., Mullaly, S. C., & Aparicio, S. (2014). The omics of triple-negative breast cancers. *Clinical chemistry*, *60*(1), 122-133.

Yu, B., O'Toole, S. A., & Trent, R. J. (2015). Somatic DNA mutation analysis in targeted therapy of solid tumours. Translational pediatrics, 4(2), 125.

Yadav, N., & Chandra, D. (2013). Mitochondrial DNA mutations and breast tumorigenesis. *Biochimica et Biophysica Acta (BBA)-Reviews on Cancer*, *1836*(2), 336-344.

Yarham, J. W., Elson, J. L., Blakely, E. L., McFarland, R., & Taylor, R. W. (2010). Mitochondrial tRNA mutations and disease. *Wiley Interdisciplinary Reviews: RNA*, *1*(2), 304-324.

Yin, C., Li, D. Y., Guo, X., Cao, H. Y., Chen, Y. B., Zhou, F., ... & Yang, H. S. (2019). NGS-based profiling reveals a critical contributing role of somatic D-loop mtDNA mutations in HBV-related hepatocarcinogenesis. *Annals of Oncology*, *30(6), 953-962*.

Yip, C. H., Bhoo Pathy, N., & Teo, S. H. (2014). A review of breast cancer research in Malaysia. *Med J Malaysia*, *69*(suppl A), 8-22.

Yuan, Y., Ju, Y. S., Kim, Y., Li, J., Wang, Y., Yoon, C. J., ... & Xu, Y. (2020). Comprehensive molecular characterization of mitochondrial genomes in human cancers. *Nature genetics*, *52*(3), 342-352.

Yun, H. R., Jo, Y. H., Kim, J., Shin, Y., Kim, S. S., & Choi, T. G. (2020). Roles of Autophagy in Oxidative Stress. *International Journal of Molecular Sciences*, *21*(9), 3289.

Zaha, D. C. (2014). Significance of immunohistochemistry in breast cancer. *World journal of clinical oncology*, *5*(3), 382.

Zhang, C., Zhang, B., Lin, L. L., & Zhao, S. (2017). Evaluation and comparison of computational tools for RNA-seq isoform quantification. *BMC genomics*, *18*(1), 583.

Zhang, R., Nakahira, K., Guo, X., Choi, A. M., & Gu, Z. (2016). Very short mitochondrial DNA fragments and heteroplasmy in human plasma. *Scientific reports*, *6*, 36097.

Zhao, H., Yang, M., Zhao, J., Wang, J., Zhang, Y., & Zhang, Q. (2013). High expression of LC3B is associated with progression and poor outcome in triple-negative breast cancer. *Medical oncology*, *30*(1), 475.

Zhidkov, I., Nagar, T., Mishmar, D., & Rubin, E. (2011). MitoBamAnnotator: A web-based tool for detecting and annotating heteroplasmy in human mitochondrial DNA sequences. *Mitochondrion*, *11*(6), 924-928.

Zhong, F., Bi, R., Yu, B., Yang, F., Yang, W., & Shui, R. (2016). A comparison of visual assessment and automated digital image analysis of Ki67 labeling index in breast cancer. *PloS one*, *11*(2), e0150505.

Zhu, W., Qin, W., Bradley, P., Wessel, A., Puckett, C. L., & Sauter, E. R. (2005). Mitochondrial DNA mutations in breast cancer tissue and in matched nipple aspirate fluid. *Carcinogenesis*, *26*(1), 145-152.

Zou, P., Liu, L., Zheng, L. D., Payne, K. K., Manjili, M. H., Idowu, M. O., ... & Cheng, Z. (2016). Coordinated upregulation of mitochondrial biogenesis and autophagy in breast cancer cells: the role of dynamin related protein-1 and implication for breast cancer treatment. *Oxidative medicine and cellular longevity*, *2016*.

BIODATA OF STUDENT

Raevathi Omasanggar was born on 1st September 1992 in the Federal Territory of Kuala Lumpur. She was raised in Batu Arang, a heritage town in Hulu Selangor, Malaysia. She received her primary education at Sekolah Kebangsaan Batu Arang, followed by secondary education at Sekolah Menengah Kebangsaan Tuanku Abdul Rahman Batu Arang in Selangor. She was later transferred to Science Stream education in 2008 and completed examinations for Sijil Pelajaran Malaysia (SPM) and Sijil Tinggi Pelajaran Malaysia (STPM) certificates at Sekolah Menengah Kebangsaan Taman Desa, Bandar Country Homes, Rawang, Selangor. Raevathi was accepted to pursue undergraduate studies at the International Medical University (IMU), Bukit Jalil, Kuala Lumpur, Malaysia and received the Bachelor of Science (Honours) Biomedical Science, Second Class-Upper Division in 2015. Completion of the final year project in molecular biology during her undergraduate study instilled a passion to continue her career in the field of research and development. This interest influenced her to continue postgraduate studies in Master of Science in Cancer Biology and Oncology at the Faculty of Medicine and Health Science in Universiti Putra Malaysia.

PUBLICATION

Omasanggar, R., Yu, C. Y., Ang, G. Y., Emran, N. A., Kitan, N., Baghawi, A., ... & Maniam, S. (2020). Mitochondrial DNA mutations in Malaysian female breast cancer patients. Plos one, 15(5), e0233461.

UNIVERSITI PUTRA MALAYSIA

STATUS CONFIRMATION FOR THESIS / PROJECT REPORT AND COPYRIGHT

ACADEMIC SESSION : FIRST SEMESTER 2020/2021

TITLE OF THESIS / PROJECT REPORT :

INVESTIGATING INTERPLAY BETWEEN MITOCHONDRIAL DNA MUTATIONS, OXIDATIVE STRESS AND CELL DEATHS IN MALAYSIAN BREAST CANCER PATIENTS

NAME OF STUDENT :

RAEVATHI A/P OMASANGGAR

I acknowledge that the copyright and other intellectual property in the thesis/project report belonged to Universiti Putra Malaysia and I agree to allow this thesis/project report to be placed at the library under the following terms:

1. This thesis/project report is the property of Universiti Putra Malaysia.
2. The library of Universiti Putra Malaysia has the right to make copies for educational purposes only.
3. The library of Universiti Putra Malaysia is allowed to make copies of this thesis for academic exchange.

I declare that this thesis is classified as:

*Please tick (√)

☐	**CONFIDENTIAL**	(Contain confidential information under Official Secret Act 1972).
☐	**RESTRICTED**	(Contains restricted information as specified by the organization/institution where research was done).
√	**OPEN ACCESS**	I agree that my thesis/project report to be published as hard copy or online open access.