ROLES OF LIPIDS AND LIPOPROTEIN METABOLISM IN THE MECHANISM OF DRUG-RESISTANT IN DIFFERENT SUBTYPES OF BREAST CANCER

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by

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LIST OF SYMBOLS

°C	degree Celsius
Da	Dalton
g	gram (weight per unit mass)
h	hour
kcal/mol	kilocalorie per mole
µg	microgram
µL	microlitre
µm	micrometre
µM	micromolar
mL	millilitre
mm	millimetre
mM	millimolar
min	minute
M	molar
nm	nanometre
nM	nanomolar
%	percentage
U/mL	unit per millilitre
v/v	volume/volume
w/v	weight/volume

LIST OF ABBREVIATIONS

ATCC	American Type Cell Collection
BC	Breast cancer
CD36	Cluster of Differentiation 36
DMEM	Dulbecco's Modified Eagle Medium
DMSO	Dimethyl Sulfoxide
EDTA	Ethylenediaminetetraacetic acid
FBS	Fetal bovine serum
LOX-1	Lectin-like, oxidized low-density lipoprotein receptor-1
OxLDL	Oxidized low-density lipoprotein
PacR	Paclitaxel-resistant
PBS	Phosphate buffered saline
PMSF	Phenylmethylsulfonyl fluoride
PVDF	Polyvinylidene difluoride
RIPA	Radio-Immunoprecipitation Assay
RPMI-1640	Roswell Park Memorial Institute 1640
TamR	Tamoxifen-resistant
VLDL	Very low-density lipoprotein

PERANAN LIPID DAN LIPOPROTEIN METABOLISMA DI DALAM MEKANISMA RINTANG UBAT BAGI SUBJENIS BARAH PAYUDARA YANG BERBEZA

ABSTRAK

Kanser payudara merupakan kanser kedua paling biasa di dunia dan menjadi punca utama kematian berkaitan kanser dalam kalangan wanita. Obesiti, yang sering disertai dengan peningkatan lipid dan lipoprotein dalam peredaran darah, semakin dikenal pasti sebagai faktor yang memburukkan perkembangan penyakit serta tindak balas terhadap terapi. Dalam persekitaran yang kaya dengan lipid ini, sel kanser memperoleh akses kepada substrat metabolik yang banyak untuk menyokong pertumbuhan, kelangsungan hidup dan penyesuaian terhadap tekanan terapi. Walau bagaimanapun, mekanisma bagaimana ketidakseimbangan metabolik berkaitan obesiti menggalakkan penggunaan lipoprotein dan menyumbang kepada rintangan terapi masih belum jelas. Kajian ini bertujuan menyiasat peranan lipoprotein teroksida berketumpatan rendah (oxLDL) dan lipoprotein berketumpatan sangat rendah (VLDL) dalam rintangan ubat dan penstrukturan semula metabolisma lipid antara subjenis kanser payudara berbeza, dengan mencirikan model sel rintang ubat serta menilai kesannya terhadap daya hidup sel, pengawalaturan ekspresi gen, sifat bermetastasis dan penurunan gen secara bersasar. Model rintang ubat dibangunkan melalui penghasilan sel MCF-7 rintang terhadap tamoxifen (MCF-7-TamR) dan sel MDA-MB-231 rintang terhadap paclitaxel (MDA-MB-231-PacR) menggunakan pendedahan berperingkat dan kaedah rawatan berdenyut. Perkembangan rintangan dinilai berdasarkan kali ganda rintangan, morfologi sel dan ekspresi protein P-gp. Tindak balas sel terhadap oxLDL dan VLDL dianalisis menggunakan ujian MTS, migrasi dan

invasi. Ekspresi gen dinilai melalui PCR masa nyata, diikuti penurunan siRNA pada gen yang paling meningkat. Komposisi lipid dianalisis menggunakan GC-MS. MCF-7-TamR menunjukkan rintangan 3.1 kali ganda terhadap tamoxifen, manakala MDA-MB-231-PacR menunjukkan rintangan 2.3 kali ganda terhadap paclitaxel. OxLDL bersifat agen yang beracun kepada sel terhadap sel induk (MCF-7) dan MCF-7-TamR, manakala VLDL merangsang pertumbuhan dalam MCF-7-TamR tetapi tidak dalam MCF-7. Bagi subjenis MDA-MB-231, kedua-dua lipoprotein memberikan kesan perencatan yang lebih kuat terhadap daya hidup sel rintang ubat. OxLDL meningkatkan keupayaan migrasi dan invasi MCF-7-TamR, manakala VLDL menunjukkan kesan perencatan. Analisis gen mendedahkan peningkatan ketara *IGF1R*, *SREBF1*, *S100P* dan *CCND1* dalam sel yang mempunyai reseptor estrogen yang didedahkan kepada oxLDL atau VLDL, manakala hanya *CCND1* yang diturunkan secara signifikan dalam MDA-MB-231-PacR. Penurunan ekspresi *IGF1R* mengurangkan daya hidup sel MCF-7/oxLDL, MCF-7-TamR/oxLDL dan MCF-7-TamR/VLDL, tetapi meningkatkan daya hidup dalam MCF-7/VLDL. Selain itu, penurunan ekspresi *IGF1R* menurunkan paras kolesterol dan asid lemak dalam MCF-7/oxLDL, manakala paras ini meningkat dalam MCF-7-TamR/oxLDL dan MCF-7/VLDL. Kesimpulannya, penemuan ini mendedahkan peranan baharu VLDL dalam menyokong pertumbuhan sel rintang tamoxifen serta memperlihatkan peralihan aktiviti penyebab kanser oleh oxLDL daripada mendorong penghasilan lipid dalam MCF-7 induk kepada meningkatkan isyarat pertumbuhan dalam sel rintang ubat. Lebih penting lagi, kajian ini mengenal pasti kaitan mekanistik baharu antara pengambilan lipoprotein dan ekspresi berlebihan *IGF1R* dalam kanser payudara yang mempunyai reseptor estrogen, sekali gus menonjolkan kelemahan metabolik yang berpotensi dimanfaatkan untuk intervensi terapeutik.

ROLES OF LIPIDS AND LIPOPROTEIN METABOLISM IN THE MECHANISM OF DRUG-RESISTANT IN DIFFERENT SUBTYPES OF BREAST CANCER

ABSTRACT

Breast cancer is the second most common cancer worldwide and a leading cause of cancer-related mortality in women. Obesity, often accompanied by elevated circulating lipids and lipoproteins, is increasingly recognised as a factor that worsens disease progression and therapy response. In this lipid-rich environment, cancer cells gain access to abundant substrates that can be exploited to support growth, survival and adaptation under therapeutic stress. However, the mechanisms by which obesity-associated metabolic dysregulation promotes lipoprotein utilisation and contributes to therapy resistance remain unclear. This study aims to investigate the roles of oxidised low-density lipoprotein (oxLDL) and very low-density lipoprotein (VLDL) in drug resistance and lipid metabolic reprogramming across different breast cancer subtypes, by characterising resistant cell models and examining their effects on cell viability, gene expression regulation, metastatic behaviour and targeted gene knockdown. Drug-resistant models were established by developing tamoxifen-resistant MCF-7 (MCF-7-TamR) and paclitaxel-resistant MDA-MB-231 (MDA-MB-231-PacR) cells through stepwise drug exposure and pulsed treatment, respectively. Resistance development was evaluated by fold resistance, cell morphology and P-gp expression. Cellular responses to oxLDL and VLDL were assessed using MTS, migration and invasion assays. Gene expression was analysed by real-time PCR, followed by siRNA knockdown of the most upregulated gene. Lipid composition was measured using GC-MS. MCF-7-TamR exhibited a 3.1-fold resistance to tamoxifen, while MDA-MB-231-

PacR showed a 2.3-fold resistance to paclitaxel. OxLDL was cytotoxic to parental cells (MCF-7) and MCF-7-TamR, whereas VLDL selectively promoted growth of MCF-7-TamR cells. In MDA-MB-231 subtype, both lipoproteins exerted stronger inhibitory effects on viability of resistant cells. OxLDL enhanced migration and invasion of MCF-7-TamR, whereas VLDL showed inhibitory effect. Gene analysis revealed significant change of *IGF1R*, *SREBF1*, *S100P* and *CCND1* expression in ER-positive cells exposed to oxLDL or VLDL, while only *CCND1* was significantly downregulated in MDA-MB-231-PacR. *IGF1R* knockdown reduced viability of MCF-7/oxLDL, MCF-7-TamR/oxLDL and MCF-7-TamR/VLDL cells, but increased viability of MCF-7/VLDL. Furthermore, *IGF1R* silencing reduced cholesterol and fatty acid levels in MCF-7/oxLDL, while elevating these metabolites in MCF-7-TamR/oxLDL and MCF-7/VLDL. In conclusion, these findings reveal a novel role of VLDL in supporting tamoxifen-resistant growth and uncover a shift in oxLDL's oncogenic activity from driving lipid synthesis in parental MCF-7 to enhancing proliferative signalling in resistant cells. Importantly, this study identifies a new mechanistic link between lipoprotein uptake and *IGF1R* overexpression in ER-positive breast cancer, highlighting potential metabolic vulnerabilities that may be exploited for therapeutic intervention.

CHAPTER 1

INTRODUCTION

1.1 Background

Breast cancer remains a major global health concern, ranking as the second most common cancer worldwide. In 2022, 2.3 million new diagnoses and 666,000 deaths were reported (Bray *et al.*, 2024), with mortality projected to rise to 1 million by 2040 (Arnold *et al.*, 2022). Among the four major molecular subtypes of breast cancer, Luminal A is the most prevalent, while triple-negative breast cancer (TNBC) is recognised as the most aggressive (Carvalho *et al.*, 2025). Despite current advancements in early detection and therapeutic strategies, approximately 25-30% of breast cancer patients still experience disease recurrence and mortality due to metastasis and treatment failure (Courtney *et al.*, 2022).

A major challenge in breast cancer treatment is drug resistance, which occurs when cancer cells no longer respond effectively to therapy, leading to cancer relapse and poor prognosis. Drug resistance is responsible for more than 90% of cancer-associated mortality (Gautam *et al.*, 2025). It can be driven by various mechanisms, including increased drug efflux, drug inactivation, epigenetic alterations, epithelial–mesenchymal transition (EMT), the tumour microenvironment, DNA repair processes, lipid metabolic reprogramming, the expansion of cancer stem cells and evasion of the immune response (Emran *et al.*, 2022). Tumour heterogeneity further complicates the treatment. Breast cancer exhibits both inter-tumour heterogeneity and intra-tumour heterogeneity (Fumagalli *et al.*, 2021), which makes it difficult to find therapies that target all tumour cells. Tumour heterogeneity increases the risk of drug resistance, as

some cells may not rely on the targeted pathway or can adapt through high mutation rates and plasticity (Lüönd *et al.*, 2021).

Among the drug resistance mechanisms, lipid metabolic reprogramming is an area that is increasingly explored recently. While glucose is considered the primary energy source for rapidly proliferating tumours, growing evidence indicates that lipid metabolism is significantly upregulated throughout various stages of cancer progression (Martin-Perez *et al.*, 2022). To survive in a hypoxic and nutrient-deprived microenvironment, tumour cells not only elevate glucose uptake and rely more on aerobic glycolysis, but also reprogram their lipid metabolism to support malignancy. This metabolic shift involves enhanced lipid uptake, increased *de novo* lipid synthesis, elevated fatty acid oxidation (FAO) and greater lipid storage capacity (Jin *et al.*, 2023a). Increased FAO promotes resistance to tamoxifen in ER-positive breast cancer cells (Jiang *et al.*, 2023). Furthermore, tamoxifen-resistant breast cancer cells often exhibit lipid accumulation and increased activity of lipid-related enzymes such as fatty acid synthase (FASN) and acetyl-CoA carboxylase (ACC) (Wang *et al.*, 2024).

It is well established that cancer cells are highly adaptable and can shift their metabolic pathways based on the tumour microenvironment. This metabolic plasticity allows them to survive in harsh environments, continue proliferating and evade therapeutic interventions (Baghban *et al.*, 2020). Besides lipolysis, cancer cells also utilize exogenous lipid sources, including lipoproteins such as VLDL and LDL, to meet their elevated lipid demand. These lipoproteins provide essential fatty acids and cholesterol that cancer cells need for rapid membrane production, energy storage and signalling pathways to support their accelerated growth and proliferation. Besides serving as an alternative source of energy, lipids also involve in promoting metastasis

by influencing cellular signalling, epigenetic regulation and membrane composition (Martin-Perez *et al.*, 2022).

This study provides novel insights into how exposure to oxLDL and VLDL influences lipid metabolic reprogramming and oncogenic signaling in drug-resistant breast cancer cells, which is an area that is still not well elucidated. Previous research on oxLDL and VLDL in cancer has primarily focused on their association with cancer risk, tumour growth and their ability to induce metastasis (Debik *et al.*, 2022; Jung *et al.*, 2020; Lu *et al.*, 2017; Mosapour *et al.*, 2020b). In this study, the significance of alternative lipid acquisition from exogenous sources and potential subtype-specific metabolic adaptations were explored. Current findings may offer a new perspective on the metabolic flexibility of resistant cancer cells and contribute to the identification of novel metabolic biomarkers. In future applications, this research could support the development of lipid-targeted therapeutic strategies or combination treatments to overcome drug resistance in breast cancer.

1.2 Problem statement

Drug resistance accounts for approximately 90% of cancer-related deaths (Łukasiewicz *et al.*, 2021). It is a major barrier to successful breast cancer treatment, contributing to disease relapse and poor prognosis. More than half of advanced estrogen receptor (ER)-positive breast cancer exhibit *de novo* resistance to tamoxifen, while approximately 40% of cases develop acquired tamoxifen resistance during the course of treatment (Hultsch *et al.*, 2018). Evidence from clinical studies showed that prolonged paclitaxel treatment resulted in significant drug resistance in approximately 50% of TNBC patients, accompanied by tumour progression (Zhao *et al.*, 2022b). Obesity, often accompanied by secondary hyperlipidemia, has been increasingly

implicated in breast cancer progression and therapy failure (Naaman *et al.*, 2022). Elevated levels of circulating lipids and lipoproteins in obese individuals provide an abundant nutrient source that can be exploited by cancer cells to sustain growth, proliferation and survival under therapeutic stress (Zipinotti dos Santos *et al.*, 2023). The presence of abundant adipocytes facilitates breast cancer progression by supplying exogenous fatty acids that are readily absorbed by tumour cells (Wan *et al.*, 2025). Such lipid enrichment of the tumour microenvironment not only enhances proliferative and invasive potential but also promotes activation of survival pathways that diminish the efficacy of endocrine and chemotherapeutic agents (Martin-Perez *et al.*, 2022). This study hypothesises that breast cancer cells exploit the uptake of oxLDL and VLDL to overcome metabolic stress imposed by drug treatment, consequently reprogramming lipid metabolism and drug resistance pathways. Currently, the mechanisms by which obesity-associated metabolic dysregulation facilitate lipoprotein utilisation and confer resistance remain poorly understood. Addressing this gap is essential to understand how lipid-rich tumour microenvironments influence therapeutic outcomes and to identify potential metabolic vulnerabilities for overcoming resistance.

1.3 Main objective

To investigate the roles of oxLDL and VLDL in drug resistance mechanism and lipid metabolism pathways across different breast cancer subtypes.

1.4 Specific objectives

1. To assess the characteristic features of tamoxifen-resistant MCF-7 and paclitaxel-resistant MDA-MB-231 cell lines from their respective parental cells.

2. To evaluate the cytotoxicity of oxLDL and VLDL on breast cancer cells.
3. To investigate the regulating roles of oxLDL and VLDL on genes involved in drug resistance, lipid metabolism and the cell cycle in breast cancer cells.
4. To examine the impact of oxLDL and VLDL on migration and invasion of breast cancer cells.
5. To determine whether genes knockdown affects lipid metabolism and the viability of breast cancer cells.

CHAPTER 2

LITERATURE REVIEW

2.1 Breast cancer epidemiology

Breast cancer occurs when cells within the breast undergo unregulated growth and division, forming an abnormal cluster of tissue known as a tumour. Breast cancer is a significant global burden and a serious threat to women's lives today. Based on the most recent report from the International Agency for Research on Cancer (IARC), breast cancer was the second most common type of cancer worldwide in 2022, with around 2.3 million new cases reported. Lung cancer was the most commonly diagnosed cancer in 2022, accounting for 12.4% of all cancers globally. This was followed by breast cancer (11.6%), colorectal cancer (9.6%), prostate cancer (7.3%) and stomach cancer (4.9%). The highest incidence rates of breast cancer were reported in France, Australia/New Zealand, Northern America and Northern Europe, where the rates were four times higher than those in South-Central Asia and Middle Africa (Bray *et al.*, 2024).

In Malaysia, breast cancer was the most common type of cancer reported in 2020 (17.3%), followed by lung (10.6%) and colon cancer (7.8%). Between the three major ethnics in Malaysia, breast cancer was most commonly detected among Chinese (40.7%), followed by Indians (38.1%) and Malays (31.5%) (Azizah *et al.*, 2019). Luminal A was the most common breast cancer subtype among all ethnic groups. However, despite having a lower overall incidence of breast cancer, Malay women exhibited a higher proportion of aggressive subtypes, including triple-negative and HER2-enriched breast cancer (MK *et al.*, 2021).

Globally, breast cancer ranks as the fourth major cause of cancer mortality. It was estimated that 666,000 women died from breast cancer in 2022, which accounts to 6.9% of cancer deaths among women. Fiji, Western Africa and Micronesia/ Polynesia had the highest mortality rates (>20 per 100,000 females) while mortality rates in most other world regions range between 10 and 15 per 100,000 females (Figure 2.1). The global burden of breast cancer is predominantly concentrated among women aged 50 and above, who account for over 70% of all new cases and 81% of all deaths (Arnold et al., 2022).

The higher rate of breast cancer incidence in transitioned countries compared to transitioning countries are due to a greater prevalence of various reproductive and lifestyle risk factors (Bray *et al.*, 2024). Recognised non-modifiable risk factors for developing breast cancer include age, family history, and menstrual and reproductive factors (Table 2.1). Modifiable risk factors are such as alcohol consumption, diet, physical activity and body weight. Women, whose mother or sister has a breast cancer, are prone to this disease. Reproductive factors such as early menstruation, late menopause, having the first pregnancy at an older age and having fewer children can increase the risk of breast cancer (Heer *et al.*, 2020). Additionally, excessive alcohol consumption and a high intake of dietary fats can also elevate the risk (Sun *et al.*, 2017). The likelihood of developing breast cancer rises in post-menopausal women who are obese, with this association being particularly notable among women from Asia, North America, Africa and Europe (Dehesh *et al.*, 2023).

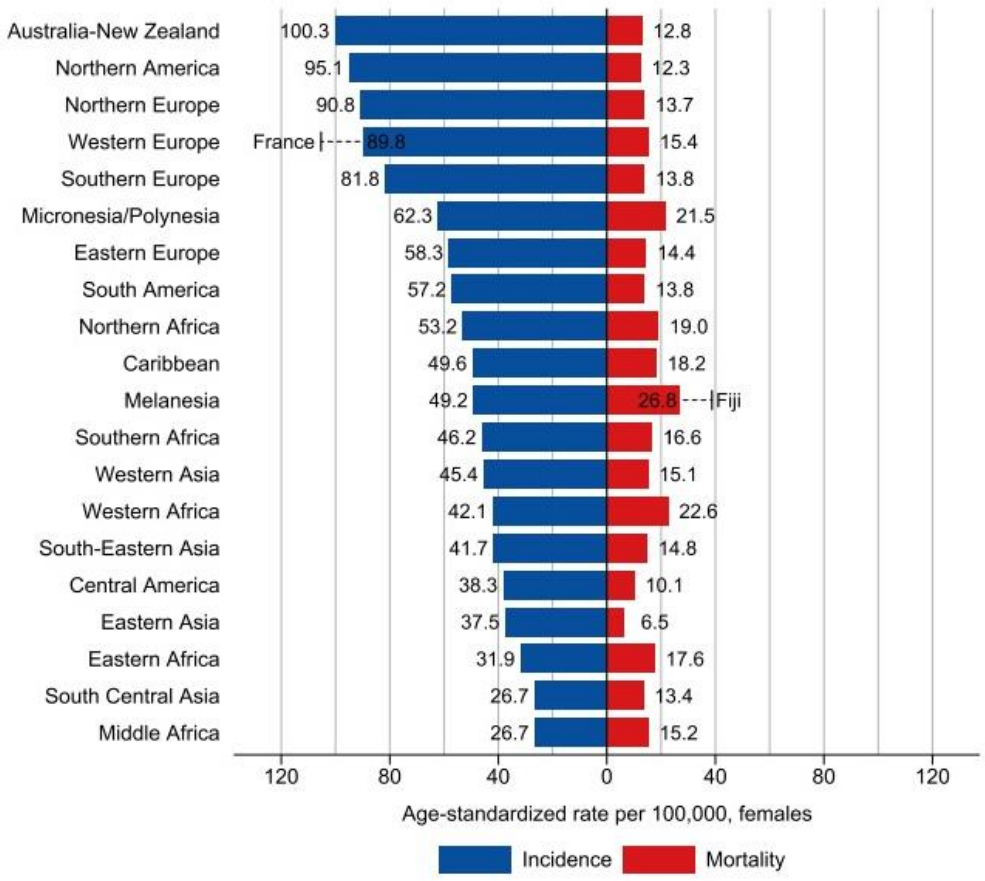

Figure 2.1: Bar chart of the region-specific incidence and mortality age-standardized rates for female breast cancer in 2022. Rates are shown in descending order of the world age-standardized rate. Source: GLOBOCAN 2022.

Table 2.1: Risk factor for breast cancer. Adapted from Eskandari *et al*., 2020.

Risk factor	**High risk group**
Age	Elderly
Reproductive risk factors	
Age at menarche	Menarche before age 11
Age at menopause	Menopause after age 54
Age at first pregnancy	Nulliparous or first child in early 40s
Lifestyle factors	
Diet	High intake of saturated fat
Body weight (postmenopausal)	Body mass index > 35
Alcohol	Excessive intake
Hormonal status	
Oral contraceptives	Current use
Hormone replacement therapy	Use for more than 10 years
Radiation	Abnormal exposure after age 10
Family history	Breast cancer in first degree relative when young

2.2 Molecular subtypes of breast cancer

Based on their pathological features, breast cancer can be non-invasive, invasive or metastatic. It consists of four molecular subtypes, which differ in terms of their expression of estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor receptor 2 (HER2) and also Ki-67 index (a proliferation marker). The gene expression profiling divides breast cancer as Luminal A (ER+/PR+/HER2-/low Ki-67), Luminal B (ER+/PR-/+/HER2-/+/high Ki-67), HER2-overexpressed (ER-/PR-/HER2+) and triple negative breast cancers (ER-/PR-/HER2-) (Goldhirsch *et al.*, 2011; Tsang *et al.*, 2020). The Luminal B subtype is more invasive and aggressive compared to Luminal A (Dai *et al.*, 2017). High Ki-67 levels are more commonly expressed in Luminal B, triple-negative and HER2-enriched tumours. This feature is useful to distinguish between Luminal A and Luminal B subtypes in ER+/HER2– breast cancer (Al-Zawi *et al.*, 2021).

Many population-based studies highlighted similar incidence trends; Luminal A is the most frequent molecular subtype encountered followed by Luminal B, HER2-overexpressed and basal-like subtypes (Pandit *et al.*, 2020). Luminal A and B are ER-positive cancers but the Luminal B subtype tend to have a worse prognosis than Luminal A (Johnson *et al.*, 2021). The HER2-overexpressed breast cancer is characterized by the overexpression of HER2/HER2 signaling-associated genes and comprises of 15% of all invasive breast cancers. Basal-like breast cancers show triple negative phenotype (lack of ER, PR and HER2-related genes) and have poor prognosis (Tsang *et al.*, 2020). The classification of normal-like breast cancer as a distinct subtype is still debated. Some researchers argue that these tumours may appear as a separate subtype due to technical issues, such as significant contamination with normal tissue during the microarray process. The absence of clear clinical significance and

specific treatment responses for these tumours further supports this view (Yersal *et al.*, 2014). The unique features of each subtype are described in Figure 2.2.

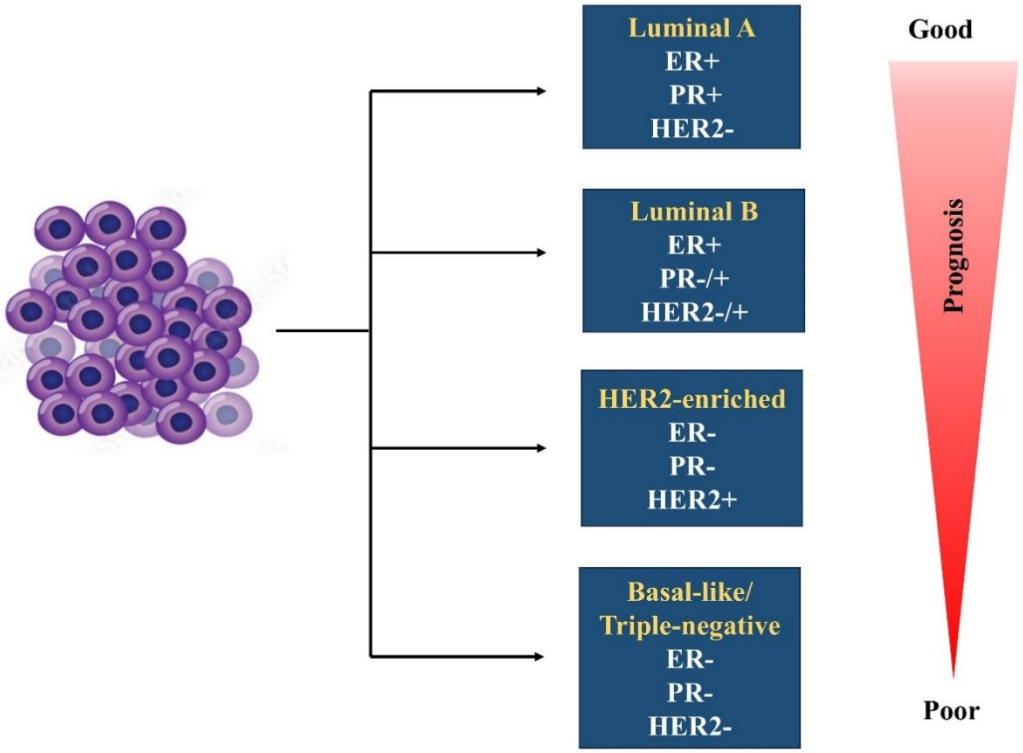

Figure 2.2: Characteristics of breast cancer molecular subtypes.

The prevalence of molecular breast cancer subtypes differs by race and ethnicity. Luminal A tumours are more prevalent among non-Hispanic white women compared to other ethnic groups. In contrast, basal-like/triple negative tumours are more common among non-Hispanic Black, African American and Hispanic women, particularly before menopause (Łukasiewicz *et al.*, 2021). In *in vitro* studies, examples of common cell lines used for breast cancer research include MCF-7 for luminal A, BT474 for luminal B, MDA-MB-453 for HER2-overexpressed and MDA-MB-231 for triple negative (Holliday *et al.*, 2011) (Dai *et al.*, 2017). Luminal tumours have better

prognosis and luminal cell lines are less aggressive, as compared to triple negative tumours and cell lines (Dai *et al.*, 2017).

2.3 Current breast cancer treatment modalities

The molecular subtypes of breast cancer described above provide valuable insights into new treatment strategies and patient stratifications which are useful for management of breast cancer patients. The approach to treating breast cancer typically involves a combination of surgical, radiation, and/or medical treatments (Łukasiewicz *et al.*, 2021). The specific approach depends on several factors, including the stage and subtype of the cancer, the patient's overall health and personal preferences and any other medical conditions (Burguin *et al.*, 2021).

The primary objectives of treating nonmetastatic breast cancer are removing the tumour from the breast and nearby lymph nodes and reducing the risk of metastatic recurrence. The local treatment approach involves surgically removing the tumour and lymph nodes, along with postoperative radiation therapy when appropriate (Waks *et al.*, 2019). The most common surgeries for breast cancer include lumpectomy, which involves removing the tumour along with a margin of surrounding tissue, and mastectomy, which entails the removal of the entire breast (Admoun *et al.*, 2021). In some cases, sentinel lymph node biopsy or axillary lymph node dissection may also be performed to determine if the cancer has spread to the lymph nodes. The current popular method for treating localized breast cancer is breast conservation surgery (Matsen *et al.*, 2013). Before undergoing the procedure, neoadjuvant therapy is administered to reduce tumour size. Following the surgery, adjuvant therapy is usually given to complete the recovery and minimise the chance of metastasis (Shien *et al.*, 2020). Radiation therapy is often administered after surgery to eliminate any

remaining cancer cells and reduce the risk of local recurrence. It involves exposing the cancer cells to high levels of radiation (Gray *et al.*, 2019).

The treatment of metastatic breast cancer varies based on its subtype, with the aim of extending survival and providing relief from symptoms. For metastatic HR+/ERBB2- breast cancer, early treatment usually involves endocrine therapy with a CDK 4/6 inhibitor like abemaciclib, palbociclib or ribociclib in the first or second line. Chemotherapy is used when hormonal treatments no longer work (Waks *et al.*, 2019). Studies show that sequential single-agent chemotherapy is just as effective as combination chemotherapy, with fewer side effects and better quality of life for patients. Therefore, single-agent sequential chemotherapy is the standard treatment (Al Sukhun *et al.*, 2024). The same approach is used for metastatic triple negative breast cancer (TNBC) , where chemotherapy is the only option for patients who do not have BRCA1/2 mutations (Waks *et al.*, 2019).

Hormonal therapy, also known as endocrine therapy, is used to block the effects of hormones that can drive the growth of hormone receptor-positive breast cancer. The most common types of hormonal therapy include selective estrogen receptor modulators (SERMs), selective modulators estrogen receptor degraders (SERDs) and aromatase inhibitors (AIs) (El Sayed *et al.*, 2019). Targeted therapy is used to target specific genetic mutations that drive the growth of cancer cells. Drugs such as trastuzumab (Herceptin®) and pertuzumab (Perjeta®) are used to target breast cancers that over-express the HER2 protein (Swain *et al.*, 2023). Immunotherapy is a relatively new approach to treating cancer and is still being studied for its effectiveness in treating breast cancer. Pembrolizumab and atezolizumab antibodies have been evaluated in numerous clinical trials, where they showed clinical efficacy and safety in patients with TNBC (Tong *et al.*, 2018).

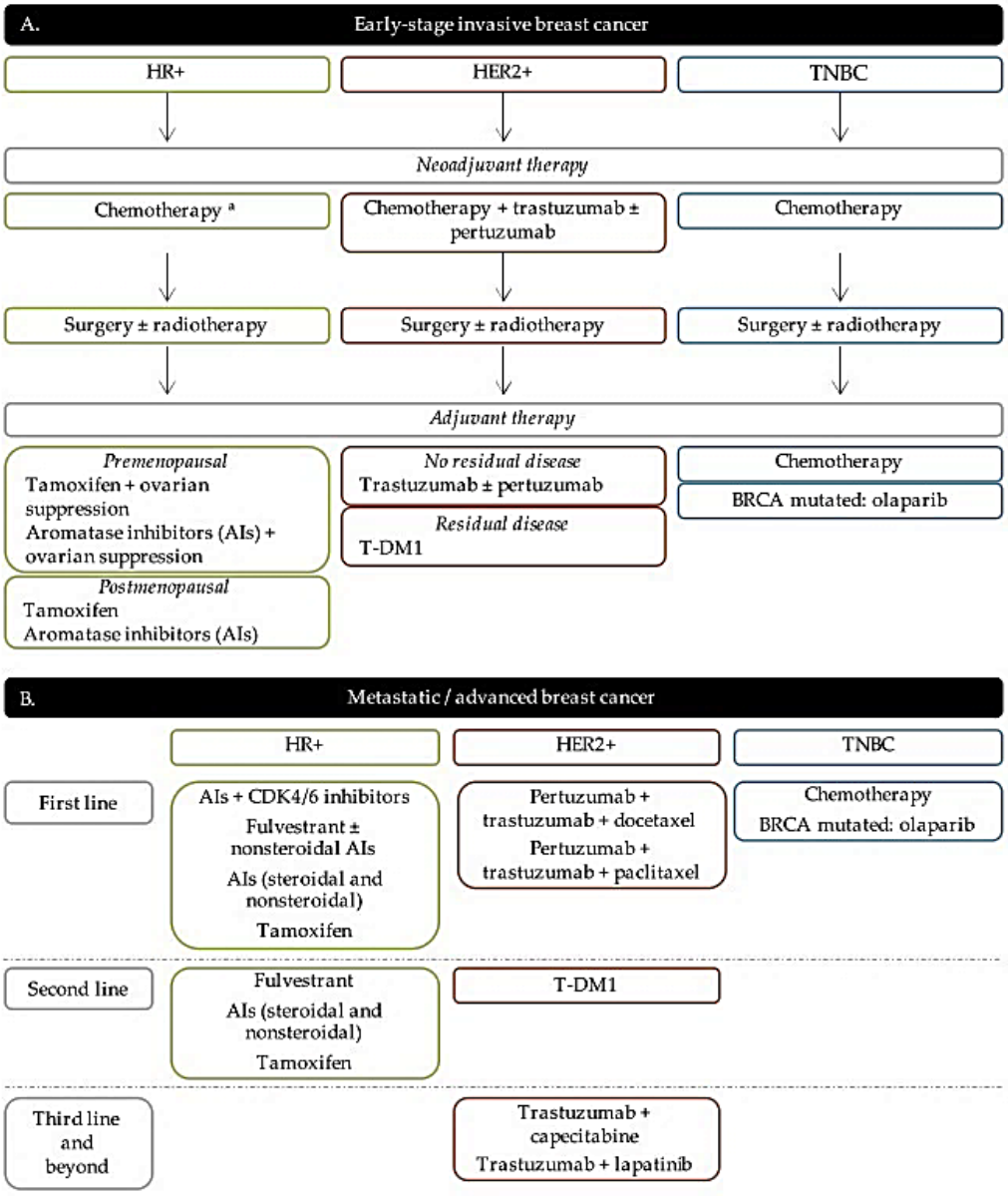

Figure 2.3: Flow diagram for personalized treatment of breast cancer. (A) Early-stage breast cancer. (B) Metastatic/ advanced breast cancer. [a]Neoadjuvant chemotherapy for HR+ breast cancer patients is not systematic. It is primarily given to patients with luminal B breast cancer and/or older breast cancer patients. HR+: hormone receptors positive; HER2+: human epidermal growth factor receptor 2 positive; TNBC: triple-negative breast cancer; AIs: aromatase inhibitors; T-DM1: trastuzumab-emtansine. Source: Burguin *et al*., 2021.

Cancer vaccines are a type of immunotherapy that trains the immune system to identify and destroy cancer cells by making it aware of their characteristics.

Vaccination is an emerging approach to prevent recurrence in high-risk breast cancer patients, especially those with TNBC (Burguin *et al.*, 2021). Recent advancements in cancer vaccines include the development of mRNA vaccine technology, inspired by the progress made with COVID-19 vaccines, and personalized vaccines that target neoantigens. Currently, only one approved cancer vaccine is available on the market (WGc-043), which specifically targets Epstein-Barr virus (EBV)-related cancers. All other cancer vaccine products are still undergoing pre-marketing clinical studies (Fan *et al.*, 2023b). The WGc-043 vaccine is designed to treat various malignancies associated with EBV, such as nasopharyngeal carcinoma, natural killer T-cell lymphoma, lung cancer, liver cancer and breast cancer (Shechter *et al.*, 2022). The integration of powerful adjuvants, innovative delivery methods and emerging gene-editing technologies like CRISPR/Cas9 holds significant potential in revolutionising the development of cancer therapeutic vaccines in the future (Hargrave *et al.*, 2023; Ahmad *et al.*, 2023).

2.4 Breast cancer recurrence: variations among subtypes

Challenges in treating breast cancer include managing resistance to treatments and the recurrence of the disease. Despite successful resection or chemotherapeutic treatment, patients with advanced cancer have poor survival rates mainly due to tumour relapse and metastasis (Mitra *et al.*, 2015). After many years of survival from breast cancer, there is possibility that the cancer can come back, either as local recurrence or distant recurrence. The pattern varies among different subtypes of breast cancer. TNBC has increased likelihood of recurrence especially within the initial 5 years after diagnosis and are prone to develop distant recurrence in the brain as well as visceral metastases. Higher rate of distant recurrence occurred in TNBC patients,

with mean time of recurrence of 2.6 years as opposed to 5 years in receptor-positive breast cancer patients (Deepak Singh *et al.*, 2021). Pogoda *et al*., (2013) reported a similar pattern of recurrence. Relapse in TNBC patients was at peak during the first 3 years and did not change significantly thereafter. Distant metastases in lung was high in TNBC cancer patients, besides characteristics of local recurrence in the ipsilateral lymph nodes (Wu *et al.*, 2016).

Moreover, patients with triple-negative cancers experienced shorter time of local recurrence as compared to ER/PR/HER-2 positive patients, with respective mean times of 2.8 and 4.2 years (Deepak Singh *et al.*, 2021). Recent findings showed that TNBC patients had shortest time to recurrence (TTR) of 26.9±28.5 months, followed by HER+ (34.3±21.8 months), Luminal B (48.4±41.1 months) and Luminal A (56.0±41.3 months). The study also concluded that estrogen receptor positivity, progesterone receptor positivity, invasive lobular carcinoma and receiving endocrine therapy predicted longer TTR (Courtney *et al.*, 2022).

2.5 Lipids and lipoproteins

Lipids are important for various biological functions. They are hydrophobic or amphiphilic molecules that function as structural components of cell membranes. Lipids can act as insulators in the body. Besides that, they involve in cell signalling and communication as well as provide cushioning and protective layer for organs. During their biosynthesis process, lipids undergo complex biochemical transformations, making their structure more complex than that of carbohydrates and proteins (Guo *et al.*, 2020). Simple lipids are the esters of fatty acids with alcohols. These include the triglycerides (triacylglycerols) and the waxes. Compound lipids are complex lipids that contain additional groupings such as phosphoric acid

(phospholipids), sugars (glycolipids) or proteins (lipoproteins) beside fatty acid and alcohol. Derived lipids are formed from hydrolysis of simple and compounds lipids. Fatty acids, steroids, eicosanoids and ketone bodies are examples of derived lipids.

Lipoproteins are particles made of proteins and lipids. Since lipids are water-insoluble, they must attach to proteins in order to be efficiently transported throughout the circulatory system. Lipoprotein particles are composed of a central hydrophobic core containing non-polar lipids, primarily cholesteryl esters and triglycerides. This core is enveloped by a hydrophilic outer layer, which includes phospholipids, free cholesterol and apolipoproteins (Figure 2.4).

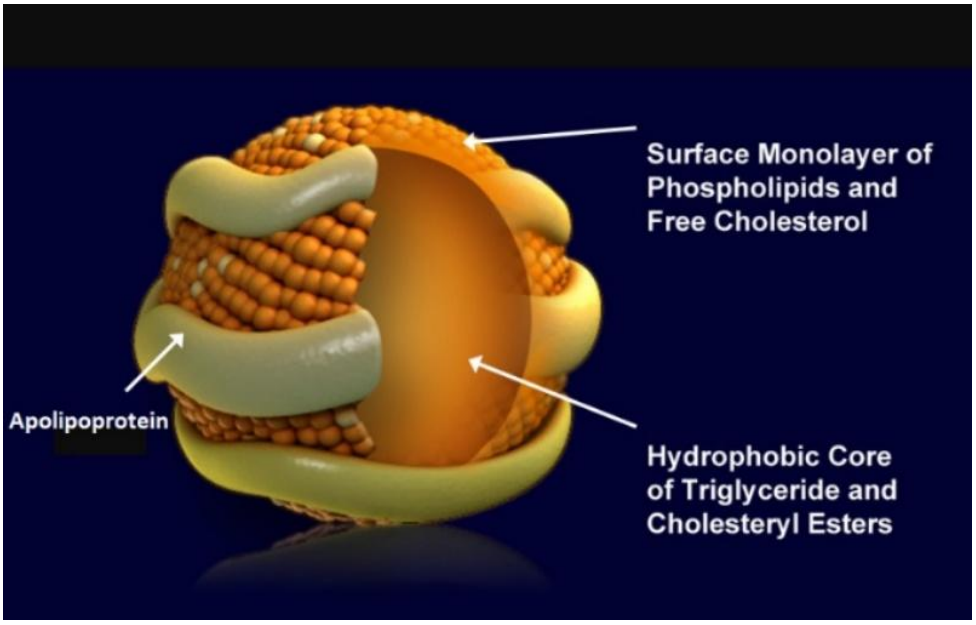

Figure 2.4: Structure of a lipoprotein molecule. Source: Feingold, 2022.

Plasma lipoproteins can be divided into several types based on their size, lipid composition and apolipoproteins: high-density lipoproteins (HDL), low-density lipoproteins (LDL), very low-density lipoproteins (VLDL), intermediate-density

lipoproteins (IDL), chylomicrons and chylomicron remnants. Chylomicrons are the largest and least dense particles, primarily transporting dietary triglycerides and cholesterol from the intestine to peripheral tissues. VLDL, produced by the liver, delivers endogenously synthesized triglycerides to peripheral tissues and its metabolism generates IDL and subsequently LDL. LDL, often referred to as "bad cholesterol," is the major cholesterol carrier in plasma and delivers cholesterol to cells via the LDL receptor. Excessive LDL accumulation contributes to atherosclerosis. In contrast, HDL, or "good cholesterol," mediates reverse cholesterol transport by shuttling excess cholesterol from peripheral tissues back to the liver for excretion. HDL also exerts antioxidant and anti-inflammatory effects. The characteristics of each type of lipoprotein are described in Table 2.2.

Table 2.2: Characteristics of different types of lipoproteins.

Lipoprotein	Density (g/ml)	Size (nm)	Major Lipids	Major Apoproteins
Chylomicrons	<0.930	75-1200	Triglycerides	Apo B-48, Apo C, Apo E, Apo A-I, A-II, A-IV
Chylomicron Remnants	0.930-1.006	30-80	Triglycerides Cholesterol	Apo B-48, Apo E
VLDL	0.930-1.006	30-80	Triglycerides	Apo B-100, Apo E, Apo C
IDL	1.006-1.019	25-35	Triglycerides Cholesterol	Apo B-100, Apo E, Apo C
LDL	1.019-1.063	18- 25	Cholesterol	Apo B-100
HDL	1.063-1.210	5- 12	Cholesterol Phospholipids	Apo A-I, Apo A-II, Apo C, Apo E

2.6 Disruption of lipoprotein metabolism in obesity

Dyslipidemia is defined as an imbalance in plasma lipid and lipoprotein levels, which is closely related to the pathogenesis of obesity. Approximately 60-70 percent of obese individuals have dyslipidemia (Alshuweishi *et al.*, 2024). This metabolic imbalance is marked by elevated triglycerides, increased levels of VLDL and apolipoprotein B, reduced HDL cholesterol and higher circulating concentrations of oxidised LDL (oxLDL) (Feingold, 2022). Under oxidative stress, the oxidation of LDL occurs by the process of lipid peroxidation and LDL is modified into oxLDL. OxLDL is a key factor in obesity-related artery disease, contributing to inflammation and plaque formation by easily trapping in artery walls, damaging endothelial cells and triggering a buildup of white blood cells that transform into foam cells within the plaques. This process is a critical component of atherosclerosis, making oxLDL a significant pathological mediator in the progression of the condition (Babakr, 2025).

In obesity, VLDL metabolism is altered through multiple mechanisms involving insulin resistance, chronic inflammation and increased free fatty acid (FFA) flux to the liver, resulting in an overproduction of triglyceride-rich VLDL particles (Dilworth *et al.*, 2021). Visceral adiposity, in particular, leads to increased lipolysis and a greater supply of FFAs to the liver. This oversupply of fatty acids and triglycerides, combined with insulin's diminished ability to suppress apolipoprotein B-100 secretion, stimulates the liver to produce and secrete an excessive number of VLDL particles (Nobarani *et al.*, 2022). This ultimately contributes to the dyslipidemia seen in obese individuals, characterized by high triglycerides and low levels of beneficial HDL cholesterol. Additionally, the function of lipoprotein lipase (LPL), which clears triglycerides from VLDL, is impaired in obesity. This is due to an

increase in LPL inhibitor proteins, such as ApoC-III, leading to higher and more prolonged VLDL levels in the bloodstream (Gugliucci, 2023).

2.7 Oncogenic roles of oxLDL and VLDL

Besides their established link with obesity, epidemiological studies also demonstrated that oxLDL and VLDL are involved in cancer progression. Oxidative stress, a common feature of the tumour microenvironment, converts LDL into oxLDL. Early in the process, the LDL is considered minimally oxidised (mmLDL), with changes primarily in its lipids. As oxidation progresses, it becomes extensively oxidised, with damage also affecting the LDL's protein component, ApoB100. This oxidised form is not recognized by LDL receptor, but is instead taken up by scavenger receptors on cancer cells, primarily CD36 and LOX-1.

The uptake of oxLDL drives key cancer processes through several signalling pathways. OxLDL generates reactive oxygen species (ROS), which can damage DNA and cause cell mutations. It also promotes the release of pro-inflammatory markers that fuel oncogenic signals. Binding of oxLDL to its receptors, such as LOX-1, activates signalling pathways like NF-κB and PI3K/Akt. This increases the expression of pro-angiogenic factors (e.g., VEGF and MMP-9), which support the formation of new blood vessels needed for tumour growth (Zhang *et al.*, 2025). Growing research attention has been directed towards oxLDL and LOX-1 in various cancers, including colorectal cancer (Nakashima-Nakasuga *et al.*, 2020), hepatocellular carcinoma (Yan *et al.*, 2019), gastric cancer (Ma *et al.*, 2019) as well as breast cancer (Pucci *et al.*, 2019). Additionally, elevated oxLDL serum level was reported among stage II and III of breast and ovarian cancer patients (Mayengbam *et al.*, 2021). *In vitro*, oxLDL induced proliferation of MCF10A cell line through miR-21 activation, consequently

inducing PI3K/Akt pathway (Deng *et al.*, 2022). LOX-1, a receptor primarily involved in the uptake of oxLDL, was upregulated in around 70% of breast cancer cases. LOX-1 expression also has been reported to correlate with both tumour grade and stage (Pucci *et al.*, 2019).

On the other hand, VLDL are lipoproteins abundant in triglycerides. VLDL is primarily composed of approximately 90% lipids and 10% proteins. Within its lipid content, about 70% constitutes triglycerides, while the remaining portion consists of cholesterol esters and fatty acids (Dashti *et al.*, 2011). VLDL production is influenced by dietary intake, with levels rising after meals. Excessive nutrition or a high-fat diet can lead to an increase in VLDL secretion, especially in individuals with hypertriglyceridemia (Huang *et al.*, 2022). In addition to lipid transport, VLDL also contributes to nitric oxide pathways, which are crucial for vascular smooth muscle relaxation and maintaining blood pressure control (Magnifico *et al.*, 2017). Furthermore, VLDL stimulates the adrenal glands to produce aldosterone and elevates cytosolic calcium levels, which enhances phospholipase D activity (Tsai *et al.*, 2017).

Elevated circulating VLDL provides an abundant source of triglycerides, cholesterol, and phospholipids, which are essential building blocks for rapidly proliferating cancer cells. By supplying these lipids, VLDL supports membrane biosynthesis, energy production through β-oxidation, and activation of signaling pathways that promote cell survival and growth. VLDL has also been shown to modulate intracellular signaling cascades such as the PI3K/Akt/mTOR pathway, thereby enhancing cell proliferation and inhibiting apoptosis (Glaviano *et al.*, 2023). In addition, VLDL-derived lipids can alter the tumour microenvironment by stimulating inflammatory responses and oxidative stress, which further drive

malignant transformation. Importantly, VLDL uptake by VLDL receptor has been linked to increased invasive and metastatic potential in certain cancers, including breast cancer (Yang *et al.*, 2022a). Epidemiological studies also suggest that hypertriglyceridemia, often associated with elevated VLDL levels, correlates with a higher risk of cancer incidence and poorer clinical outcomes (Ma *et al.*, 2021).

Clinically, the link between plasma lipids and breast cancer has been extensively studied. In adipose-rich environments such as the breast tissue, tumour cells can receive lipids via lipolysis of nearby adipocytes or uptake of circulating lipoproteins (Lee-Rueckert *et al.*, 2023). A retrospective cohort study in southwest of China revealed that the total serum levels of total cholesterol (TC), triglycerides (TG), high density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) are significantly lower in breast cancer patients than those in normal controls. However, after chemotherapy, the levels of TC, TG, LDL-C and apolipoprotein B among the breast cancer patients were significantly higher than before chemotherapy (Li *et al.*, 2018). Recent findings also indicated no significant difference in terms of plasma lipid levels between control subjects and patients with breast cancer (Sawada *et al.*, 2023). In contrary, there was significant increase of TG, LDL-C and very low density lipoprotein-cholesterol (VLDL-C) levels in Thai women with breast cancer in comparison to controls (Laisupasin *et al.*, 2013). Breast cancer patients showed increased serum oxLDL levels as well as TC and LDL-C serum levels compared to controls (Delimaris *et al.*, 2007). Notably, breast cancer patients diagnosed with higher LDL-C levels exhibited larger tumours, increased differentiation grade and a higher proliferative rate (Rodrigues dos Santos *et al.*, 2014). Supporting this, two Mendelian randomized studies indicated a higher risk of breast cancer with increased plasma LDL-C (Nowak *et al.*, 2018; Johnson *et al.*, 2020). However, a recent systematic

review concluded that there was no significant effects of LDL-C on breast cancer risk (Amerizadeh *et al.*, 2022). It is important to note that LDL refers to the lipoprotein particle itself, whereas LDL-C and VLDL-C specifically denote the cholesterol content within LDL or VLDL particles.

2.8 Dysregulated lipid metabolism in cancer drug resistance

Lipid metabolism is a fundamental cellular process that regulates the synthesis, storage and utilization of lipids. During lipogenesis, acetyl-CoA carboxylases 1 and 2 (ACACA and ACACB) play a crucial role by converting acetyl-CoA into malonyl-CoA, a key step in the process. FASN then utilizes acetyl-CoA molecules to construct lipid carbon chains, ultimately producing palmitate (a 16-carbon fatty acid). Acetyl-CoA is also used to synthesize cholesterol. It is converted to acetoacetyl-CoA (4 carbons) before forming 3-hydroxy-3-methylglutaryl-CoA (HMG-CoA). HMG-CoA is then converted to mevalonate by the enzyme HMG-CoA reductase. During glycolysis, glucose is broken down to produce energy in the form of ATP (adenosine triphosphate) and acetyl-CoA to support cycling of the tricarboxylic acid (TCA) cycle. Mitochondrial fatty acid oxidation (FAO) provides alternative source of acetyl-CoA within the mitochondria for TCA cycle metabolism. Citrate formation occurs in the course of the TCA cycle and marks the initial stage of *de novo* lipogenesis as it is transported out of the mitochondria. Glutamate offers another avenue for acetyl-CoA production, employing reductive carboxylation to support cytosolic citrate pools via α-ketoglutarate and isocitrate, facilitated by cytosolic enzyme IDH1 and mitochondrial enzyme IDH2. Acetate and citrate can also serve as a substrate for acetyl-CoA generation through acetyl-CoA synthetase (ACSS2) and ATP-citrate lyase (ACLY), respectively.

Lipids can be transported into cells via passive diffusion, fatty acid transport proteins and fatty acid translocase/CD36. Lipids can be stored in lipid droplets (LDs) in the form of triacylglycerides (TAG) and cholesteryl esters. Gglycerol-3-phosphate acyltransferase (GPAT) combines glycerol-3-phosphate with a long-chain FA to produce lysophosphatidic acid (LPA), which is the rate-limiting step in TAG synthesis. LPA is converted to phosphatidic acid (PA) by acylglycerophosphate acyltransferase (AGPAT) and then to DAG by phosphatidic acid phosphatase (PAP). Diglyceride acyltransferase (DGAT) catalyzes the final step to produce TAG species, which can then be sequestered and stored in LDs. Figure 2.5 describes major lipid metabolism pathways within a cell.

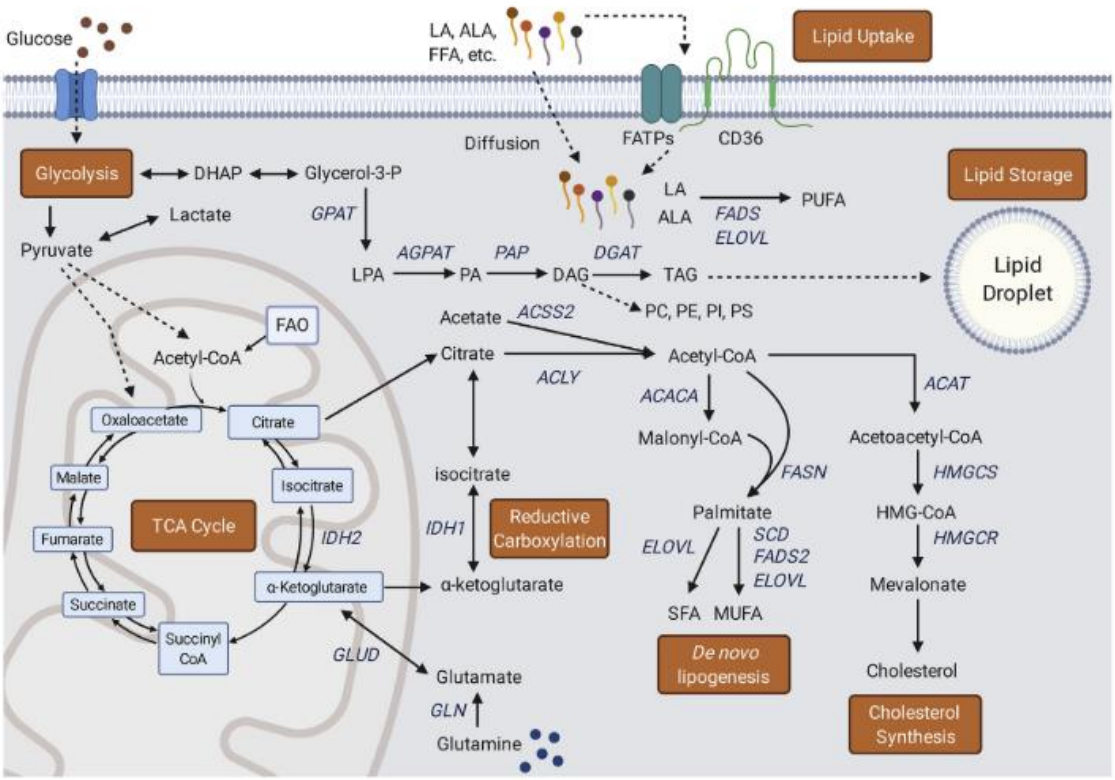

Figure 2.5: Pathways related to lipid synthesis, catabolism, uptake and storage. Source: Broadfield *et al*., 2021.

Alterations in lipid metabolism act as a cytoprotective response against oxidative stress triggered by the anticancer drugs in cancer cells. The oxidative stress induced by anticancer drugs leads to the peroxidation of lipid membranes and oxidative modifications in proteins and DNA. For instance, doxorubicin, with its anthracycline structure, generates ROS, causing DNA damage and exhibiting anticancer activity. Similarly, vinca alkaloids enhance intracellular ROS production by depleting intracellular glutathione (GSH), resulting in DNA damage (Germain *et al.*, 2020). The production of LDs counteracts membrane lipid peroxidation, leading to the reorganization of oxidised lipids and a reduction in the percentage of oxidised lipids in cell membranes (Jin *et al.*, 2020). Additionally, LDs may potentially sequester harmful molecules like ROS or lipid peroxides, providing protection against oxidative stress (Jarc *et al.*, 2019).

The saturation level of lipids influences the physicochemical properties of cell membranes. Higher levels of saturated fatty acids (SFAs) make cell membranes more resistant to ROS-dependent peroxidation and cell death by ferroptosis (Liang *et al.*, 2022). Cancer cells gain higher levels of SFAs through a process of metabolic reprogramming that enhances *de novo* fatty acid synthesis, increases lipid uptake from their environment and carefully regulates desaturation (Mallick *et al.*, 2023). Instead of relying on dietary fats like most healthy cells, tumour cells overexpress key enzymes like FASN to produce SFAs such as palmitate at an accelerated rate (Zheng *et al.*, 2021). This internal production is a crucial advantage for rapid, uncontrolled cell proliferation, as SFAs are essential building blocks for new cell membranes. In metabolically challenging environments, cancer cells also increase the expression of transporters like CD36 to scavenge and internalize lipids from surrounding tissue and a nutrient-poor microenvironment, especially during hypoxia (Munir *et al.*, 2019).