PRECLINICAL EVALUATION OF MECHANISMS UNDERLYING CHEMOTHERAPY-INDUCED OVARIAN DYSFUNCTION AND THE RESTORATIVE POTENTIAL OF MESENCHYMAL STEM CELLS IN PREMATURE OVARIAN INSUFFICIENCY

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by

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LIST OF SYMBOLS

α	Alpha
β	Beta
×g	g-force
℃	Degree Celsius
µg	Microgram
ng	Nanogram
kg	Kilogram
µl	Microlitre
ml	Millilitre
µm	Micrometre
min	Minutes
rpm	Revolutions per minute
vg	Viral genomes
%	Percentage
~	Approximately
™	Trademark

LIST OF ABBREVIATIONS

AAV	Adeno-associated virus
ADSCs	Adipose-derived stem cells
AFMSCs	Amniotic fluid mesenchymal stem cells
AKI	Acute kidney injury
Akt	Protein kinase B
AMH	Anti-Müllerian hormone
AMMSCs	Amniotic membrane-derived MSCs
AMPK	5' AMP-activated protein kinase
ARE	Antioxidant response element
BDNF	Brain-derived neurotrophic factor
bFGF	Basic fibroblast growth factor
BMSCs	Bone marrow-derived mesenchymal stem cells
BPES	Blepharophimosis-ptosis-epicanthus-inversus syndrome
CAT	Catalase
CESP-1	Corneal endothelium-specific protein
Cis	Cisplatin
CTX	Cyclophosphamide
CVD	Cardiovascular disease
DEGs	Differential expressed genes
DFO	Deferoxamine
DMEM	Dulbecco's modified eagle medium
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
DNR	Daunorubicin
Dox	Doxorubicin
DTNB	5,5'-dithiobis-2-nitrobenzoic acid
E2	Estradiol
ECM	Extracellular matrix
EDTA	Ethylenediaminetetraacetic acid
EGF	Epidermal growth factor
EPCs	Endothelial progenitor cells

ER	Estrogen receptor
EVs	Extracellular vesicles
Exos	Exosomes
Fer-1	Ferrostatin-1
FH4	Tetrahydrofolate
fMSCs	Foetal mesenchymal stem cells
FOXO	Forkhead box O
FSH	Follicle-stimulating hormone
FSHR	The follicle-stimulating hormone receptor
GCs	Granulosa cells
G-CSF	Colony-stimulating factor
GnRH	Gonadotropin-releasing hormone
GPX4	Glutathione Peroxidase 4
GWAS	Genome-wide association studies
GSH	Glutathione
GSM	Genitourinary syndrome of menopause
GSSG	Glutathione disulfide
H_2O_2	Hydrogen peroxide
hCPMSCs	Human chorionic plate-derived mesenchymal stem cells
hESC-MSCs	Human embryonic stem cell-derived MSCs
HGF	Hepatocyte growth factor
HO-1	Hemeoxygenase-1
HPG	Hypothalamic-pituitary-gonadal
HRT	Hormone replacement therapy
HSCT	Hematopoietic stem cell transplantations
HUMMR	Hypoxia up-regulated mitochondrial movement regulator
IGF	Insulin-like growth factor
IL	Interleukin
iPSCs	Induced pluripotent stem cells
JNK1	c-Jun N-terminal protein kinase 1
KEGG	Kyoto encyclopedia of genes and genomes
LIF	Leukemia inhibitory factor
MAPK	Mitogen-activated protein kinases
MDA	Malondialdehyde

MenSCs	Menstrual blood-derived endometrial stem cells
MGARP	Mitochondria localized glutamic acid-rich protein
MMP	Mitochondrial membrane potential
MSCs	Mesenchymal stem cells
MVs	Microvesicles
MT	Membrane receptor
mtDNA	Mitochondrial DNA
NAC	N-acetylcysteine
NOQ1	NAD(P)H quinone dehydrogenase 1
Nrf 2	Nuclear factor E2–related factor 2
O2·-	Superoxide anion
OSAP	Ovary-specific acidic protein
P3	Passage 3
PAI	Plasminogen activator inhibitor
PBS	Phosphate-buffered saline
PCNA	Proliferating cell nuclear antigen
PDGF	Platelet-derived growth factor
PDMSCs	Placenta-derived mesenchymal stem cells
PGCs	Primordial germ cells
PI3K	Phosphatidylinositol-3-kinase
PKA	Protein kinase A
POLG1	Polymerase gamma 1
POI	Premature ovarian insufficiency
PTEN	Phosphatase and tensin homolog deleted on chromosome 10
PUFAs	Polyunsaturated fatty acids
RFP	Red fluorescent protein
RNA	Ribonucleic Acid
ROS	Reactive oxygen species
RT-PCR	Reverse transcription polymerase chain reaction
SCF	Stem cell factor
SOD	Superoxide dismutase
StAR	Steroidogenic acute regulatory protein
SNPs	Single nucleotide polymorphisms
Tax	Paclitaxel

TBA	Thiobarbituric acid
TFR	Transferrin receptor
TGF	Tissue growth factor
TIMPs	Tissue inhibitor of metalloproteinases
TMRE	Tetramethylrhodamine ethyl ester perchlorate
TNB	2-nitro-5-mercaptopic acid
TUNEL	Terminal-deoxynucleotidyl Transferase Mediated Nick End Labeling
UCMSC	Umbilical cord mesenchymal stem cell
ucPRP	Umbilical cord blood platelet-rich plasma
VEGF	Vascular endothelial growth factor
·OH	Hydroxy radical

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PENILAIAN PRAKLINIKAL TERHADAP MEKANISMA YANG MENDASARI DISFUNGSI OVARI YANG DISEBABKAN OLEH KEMOTERAPI DAN POTENSI PEMULIHAN SEL TUNJANG MESENKIMAL DALAM KETIDAKCUKUPAN OVARI PRA-MATANG

ABSTRAK

Sungguhpun perawatan kemoterapi secara konvensional dapat menghapuskan kanser sel, malangnya ia turut memberi kerosakan kepada sel normal yang mempunyai keupayaan proliferatif yang tinggi, terutamanya boleh menyebabkan kesan toksik terhadapi ovari. Kajian ini telah merungkai mekanisma asas kerosakan ovari yang disebabkan oleh kemoterapi, dengan matlamat membuka laluan untuk pembangunan adjuvan penjagaan kesuburan bagi pesakit wanita yang menjalani rawatan kanser secara konvensional. Dalam konteks ini, sel tunjang mesenkimal vesikel ekstraselular (MSC-EV), sebagai agen terapeutik bebas sel yang mempunyai kelebihan yang ketara dalam menangani disfungsi ovari. Walau bagaimanapun, mekanisma tepat bagaimana MSC-EV mendasari ovotoksisiti yang disebabkan oleh kemoterapi masih tidak jelas. Objektif utama kajian ini adalah untuk menerangkan toksisiti dadah kemoterapi terhadap ovari, merungkai potensi mekanisma yang menyebabkan kehilangan folikel, dan menyelidiki potensi terapeutik transplantasi MenSC-EV dalam mengurangkan disfungsi ovari yang disebabkan oleh kemoterapi. Dengan menggunakan teknik hematoxylin dan eosin (HE), pewarnaan *trichrome masson*, ujian terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL), *western blotting*, pewarnaan imuohistokimia, pewarnaan imunofluoresensi, ujian daya maju sel, mikroskop elektron penghantaran, penjujukan

ribonucleic acid (RNA), ujian potensi membran mitokondria, ujian spesies oksigen reaktif lipid (ROS) , ujian glutathione (GSH) dan teknik lain, kajian ini mendapati bahawa dadah kemoterapi konvensional, iaitu siklofosfamid (CTX), paklitaksel (Tax), doksorubisin (Dox), dan cisplatin (Cis), secara signifikan mengurangkan isipadu ovari dan jumlah folikel primordia dan antral pada tikus, disertai oleh fibrosis ovari dan kurangnya simpanan ovari dalam model haiwan. Selain itu, rawatan Tax, Dox, dan Cis menyebabkan apoptosis dalam sel granulosa ovari (GC), berkemungkinan menyebabkan pengeluaran ROS yang berlebihan telah mengakibatkan kerosakan oksidatif dan kapasiti anti-oksidatif sel yang terjejas. Penyelidikan lanjut menunjukkan bahawa rawatan Cis mengaruh disfungsi mitokondria dalam GC, menyebabkan pengeluaran superoksida yang berlebihan dan mencetus peroksidasi lipid, menjurus kepada ferroptosis - penemuan yang belum pernah berlaku dalam kerosakan ovari yang disebabkan oleh kemoterapi. Terdapat juga bukti bahawa rawatan N-asetil sistein (NAC) berkesan dalam mengurangkan toksisiti yang disebabkan oleh Cis dengan mengatur tahap ROS dan meningkatkan kapasiti anti-oksidatif. Kajian ini juga membuktikan bahawa penyerapan MenSC-EV oleh sel SVOG (simian virus 40-immortalized granulosa cells) yang cedera oleh Cis meningkatkan keupayaan anti-apoptosis, menggalakkan proliferasi selular, dan mengurangkan tahap ROS intraselular, dengan demikian memberikan ketahanan terhadap sitotoksisiti yang disebabkan oleh Cis. Selain itu, rawatan MenSC-EV meningkatkan simptom kekurangan ovari pramatang (POI), sebahagiannya melalui peningkatan ekspresi mitochondria-localized glutamic acid-rich protein (MGARP). Secara keseluruhan, kajian ini mengesahkan impak gangguan kemoterapi terhadap keseimbangan hormon dan integriti ovari dalam model praklinikal. Ia mendedahkan mekanisma baharu di mana ubatan kemoterapi mengaruh ferroptosis dalam sel ovari

melalui peroksidasi lipid yang disebabkan oleh ROS dan disfungsi mitokondria, membawa kepada kematian sel ovari. Tambahan pula, kajian ini mengemukakan pandangan ke atas mekanisma molekul melalui MSC, khususnya MenSC-EV, meredakan ovotoksisiti yang disebabkan oleh Cis dengan mengatur disfungsi mitokondria. Oleh itu, pembangunan pelindung kesuburan dari perspektif tekanan oksidatif dan ferroptosis yang disebabkan oleh kemoterapi akan mengurangkan kerosakan ovari dan meningkatkan kualiti hidup pesakit kanser.

PRECLINICAL EVALUATION OF MECHANISMS UNDERLYING CHEMOTHERAPY-INDUCED OVARIAN DYSFUNCTION AND THE RESTORATIVE POTENTIAL OF MESENCHYMAL STEM CELLS IN PREMATURE OVARIAN INSUFFICIENCY

ABSTRACT

Conventional chemotherapy, while effective in eliminating cancer cells, unfortunately, inflicts collateral damage on normal cells with heightened proliferative capacity, particularly causing ovarian toxicity. This study delves into elucidating the underlying mechanisms of chemotherapeutic drug-induced ovarian damage, aiming to pave the way for the development of fertility-preserving adjuncts for female patients undergoing conventional cancer treatment. In this context, mesenchymal stem cells-extracellular vesicles (MSC-EVs), as promising cell-free therapeutic agents, demonstrate notable advantages in addressing ovarian dysfunction. However, the precise mechanisms through which MSC-EVs ameliorate chemotherapy-induced ovotoxicity remain unclear. The primary objectives of this study are to elucidate the toxicity of chemotherapeutic drugs on the ovary, unravel the potential mechanisms leading to follicle loss, and investigate the therapeutic potential of Menstrual blood-derived endometrial stem cell (MenSC)-EVs transplantation in alleviating chemotherapy-induced ovarian dysfunction. Applying haematoxylin and eosin (HE) staining, masson trichrome staining, terminal deoxynucleotidyl transferase dUTP nick end labelling (TUNEL) assay, western blotting, immuohistochemical staining, immunofluorescence staining, cell viability assay, transmission electron microscope, RNA-Sequencing, mitochondrial membrane

potential assay, lipid reactive oxygen species (ROS) assay, GSH assay and other techniques, the research revealed that conventional chemotherapeutic drugs, namely cyclophosphamide (CTX), paclitaxel (Tax), doxorubicin (Dox), and cisplatin (Cis), significantly reduced ovarian volume and the number of primordial and antral follicles, accompanied by ovarian fibrosis and diminished ovarian reserve in animal models. Moreover, Tax, Dox, and Cis treatments induced apoptosis in ovarian granulosa cells (GCs), likely attributed to excessive reactive oxygen species (ROS) production-induced oxidative damage and compromised cellular anti-oxidative capacity. Further investigations indicated that Cis treatment induced mitochondrial dysfunction in GCs, leading to superoxide overproduction and triggering lipid peroxidation, culminating in ferroptosis—an unprecedented finding in chemotherapy-induced ovarian damage. Notably, N-acetylcysteine (NAC) treatment proves effective in mitigating Cis-induced toxicity by regulating ROS levels and enhancing anti-oxidative capacity. The study also demonstrated that MenSC-EVs uptake by Cis-injured SVOG (simian virus 40-immortalized granulosa cells) cells enhanced anti-apoptotic capability, promotes cellular proliferation, and reduces intracellular ROS levels, thereby conferring resistance against Cis-induced cytotoxicity. Additionally, MenSC-EVs treatment improves premature ovarian insufficiency (POI) symptoms, partially through the up-regulation of mitochondria localized glutamic acid-rich protein (MGARP) expression. In summary, this research confirms the disruptive impact of chemotherapy on hormonal balance and ovarian integrity in preclinical models. It uncovers a novel mechanism wherein chemotherapeutic drugs induce ferroptosis in ovarian cells through ROS-induced lipid peroxidation and mitochondrial dysfunction, leading to ovarian cell death. Furthermore, the study provides insights into the molecular mechanisms through

which MSCs, particularly MenSC-EVs, mitigate Cis-induced ovotoxicity by improving mitochondrial dysfunction. Consequently, creating fertility protectants that address chemotherapy-induced oxidative stress and ferroptosis will mitigate ovarian damage and enhance the quality of life for cancer patients.

CHAPTER 1

INTRODUCTION

Chemotherapy retains its essential and irreplaceable role in the field of cancer treatment, even in the face of the progress made in targeted therapy and immune checkpoint inhibitors (Davern and Lysaght, 2020). However, widespread global attention has begun to focus on reproductive dysfunction with the use of chemotherapeutic agents. Breast cancer is the most prevalent malignancy in women of reproductive age, with over 10% of new diagnoses occurring in patients under the age of 40 (Kim *et al.*, 2016). Approximately 50% of young women experience diminished ovarian function following chemotherapy for breast cancer (Ruddy *et al.*, 2014). Unfortunately, the loss of ovarian function can result in infertility and the onset of enduring health complications stemming from premature menopause and a deficit in oestrogen, encompassing symptoms such as vasomotor disturbances, osteoporosis, sexual and cognitive dysfunction, and cardiovascular ailments (Griffiths *et al.*, 2020; Bhardwaj *et al.*, 2023). However, the underlying mechanism of chemotherapeutic drug-induced ovarian dysfunction remains unclear. Besides, there exists a scarcity of efficacious treatment modalities coupled with a relatively unfavourable prognosis concerning chemotherapy-induced ovotoxicity (Balachandren and Davies, 2017; Lambertini *et al.*, 2017; Spears *et al.*, 2019). Therefore, exploring the underlying mechanism of chemotherapeutic drug-induced ovarian dysfunction and new therapeutic strategies for chemotherapy-induced ovarian damage are urgently needed.

1.1 Ovarian structure and function

Throughout the lifespan of prenatal development to ageing, the ovary exhibits remarkable flexibility as it undergoes persistent alterations in both structure and function (Monget *et al.*, 2021). Regarding the ovary, the aforementioned alterations mainly include the initial stages of follicle formation, followed by follicular growth and atresia, steroidogenesis, oocyte maturation, as well as determinations concerning the number of mature oocytes released for fertilization and the involvement of the corpus luteum (Richards, 2018). Furthermore, human ovary ageing is a dynamic process that initiates the depletion of the initial pool of oocytes during foetal development. Throughout life, this process persists as the oocyte population gradually declines due to three fundamental events: (1) degeneration or loss of follicles, (2) formation of the corpus luteum, and (3) transformation of various cell populations from degenerating follicles and luteal structures into stromal cells (Broekmans *et al.*, 2009). The outcome of ovarian ageing is the transformation from a cyclic producer of oestrogen and progesterone, rich in follicles, to a noncyclic, low-secretory organ predominantly composed of stromal cells and producing low levels of androgens (Camaioni *et al.*, 2022).

Morphological changes occurring in the human ovary initiate from foetal life and persist throughout childhood and adulthood. Briefly, around 5 months after fertilization, the primitive ovary starts to exhibit distinguishable regions, namely the cortex and medulla (Overland *et al.*, 2023). Rudimentary follicle development and stromal cell proliferation continue during foetal development, resulting in the presence of essential tissue components required for future gametogenesis and hormone production at birth (Forabosco *et al.*, 1991; Cole *et al.*, 2006). Subsequently, in childhood, especially in the following sixth year, various modifications occur in

the ovaries, which mainly include incomplete development of follicles, the emergence of cystic follicles, localized transformation of follicular and stromal cells into luteinized structures, and progressive integration of thecal cells from degenerated follicles into the ovarian stroma (Anderson *et al.*, 2014). Consequently, based on these indispensable alterations and the growth of additional structural components, such as collagen, contributed to a substantial thirtyfold rise in ovarian weight by the onset of the first menstrual cycle (Oktem and Oktay, 2008).

The mature human ovary is composed of an outer region known as the cortex and an inner region named as the medulla (Soares *et al.*, 2015). The cortex is covered by a modified mesothelium (surface epithelium) and contains various components such as connective tissue, microvessels, follicular and luteal complexes at different stages of development, and different types of stromal cells (Wagner *et al.*, 2020). Distinguished from the cortex, the medulla primarily consists of fibrovascular tissue, nerves, and embryonic remnants like the rete ovary (O'Neill *et al.*, 2023). The structural characteristics of the adult ovary become evident after several anovulatory cycles occurring around the time of puberty. These characteristics signify the preparation of the ovary for its central event, which is ovulation (Sellix and Menaker, 2010). Ovulation encompasses the cyclic maturation of a group of follicles, the regression or atresia of all but one of these follicles, the release of the selected follicle during ovulation, the transformation of the ovulatory follicle into the corpus luteum during the cycle, and the incorporation of the corpus luteum into the corticomedullary stroma in the absence of successful fertilization (McGee and Hsueh, 2000). These ovarian morphological changes, along with the progressive decline of follicles, continue throughout the entire premenopausal period and lead to significant remodelling of the ovary (Yoshihara *et al.*, 2023). However, the periodic cycle of

these events will be disrupted when menopause approaches, and similar to puberty, the ovarian morphology reflects the occurrence of anovulatory cycles, abnormal ovulations, and inadequate formation of the corpus luteum once again (Wang *et al.*, 2020b).

1.1.1 Ovarian Follicles

In mammalian ovaries, follicles consist of an internal oocyte enveloped by granulosa cells (GCs) and external thecal cell layers. Since the day of birth, a female infant possesses approximately 1–2 million primordial follicles within her ovaries. Upon reaching menarche, the initial significant reproductive milestone, the number of follicles decreases to approximately 400,000, and this decline occurs at a relatively consistent rate of 1000 follicles per month (te Velde, 1993; Cacciottola *et al.*, 2023). Generally, the fate of each follicle is governed by both endocrine and paracrine factors. After birth, the ovarian reserve is comprised of a collection of follicles in a resting state (Gougeon, 2010). This reserve includes primordial, transitory, and small primary follicles (Figure 1.1 A, B, C). Various follicle types exhibit differences in the ratio of flattened to cuboidal GCs they harbour, and the follicular diameter is influenced by both the quantity and size of GCs. Nevertheless, there is no variation in the mean diameter of their oocyte and nucleus (Matsuda *et al.*, 2012). Additionally, these follicles do not express functional gonadotropin receptors and constitute approximately 91 to 98% of the entire ovarian follicular population (Wang *et al.*, 2017a). These follicles undergo sequential stages of primordial, primary, and secondary development before acquiring an antral cavity. In the antral stage, a majority of follicles undergo atretic degeneration, while a small subset, stimulated by cyclic gonadotropins in post-puberty, are advanced to the preovulatory stage (Baerwald *et al.*, 2012). The process of folliculogenesis initiates when

quiescent follicles (ovarian follicles that are in a dormant or inactive state) deviate from the ovarian reserve and ends with the development of a solitary dominant follicle in each menstrual cycle (Baerwald *et al.*, 2012). Briefly, this physiological progression can be subdivided into four primary stages: initiation, early follicle growth, selection of a single follicle from a cohort of selectable follicles (measuring ≥ 2 mm), and maturation of the preovulatory follicle (Yang *et al.*, 2020a).

In the growth phase, follicles undergo enlargement result of both GCs proliferation and oocyte size augmentation. The initial stage of human follicular growth mainly includes the formation of the large primary follicle (Figure 1.1 D). Subsequently, follicles undergo a transition to become secondary follicles, characterized by the presence of two or more complete layers of GC surrounding the oocyte (Figure 1.1 F). These secondary follicles are supplied by one or two arterioles, which terminate in an anastomotic network just outside the basal lamina. During this stage, certain stromal cells adjacent to the basal lamina align parallel to each other, forming the theca layer. As the follicle continues to grow, the theca layer undergoes stratification and differentiation into two distinct parts. The outer part, known as the theca externa, consists of cells that are indistinguishable from the undifferentiated theca cells. In contrast, the inner part, named the theca interna, contains fibroblast-like precursor cells that can be transformed into steroid-secreting cells, also known as epithelioid cells (Figure 1.1 H). The appearance of epithelioid cells indicates the stage at which the secondary follicle is defined as a preantral follicle (Figure 1.1 G). This classification is based on the morphological characteristics and total number of GCs existing in each follicle. Thereafter, the follicle undergoes a transition to the early antral stage (Figure 1.1 I) characterized as small fluid-filled cavities merging together, in which the follicular fluid with a composition similar to that of the blood

serum. Simultaneously, the GCs surrounding the oocyte form the structure known as the "cumulus oophorus" (Figure 1.1 J). By amassing fluid within the antral cavity and experiencing the proliferation of GCs and theca-interstitial cells, the follicle acceleratingly advances across successive developmental stages (Figure 1.1 K, L) until it obtains a size ranging from 2 to 5 mm and then is transformed into a follicle eligible for selection (Figure 1.1 M). Additionally, a challenge existed in scientific observation: The recognition of atresia in quiescent follicles is particularly difficult due to the rapid elimination of apoptotic oocytes (Figure 1.1 E) (Gougeon, 2010).

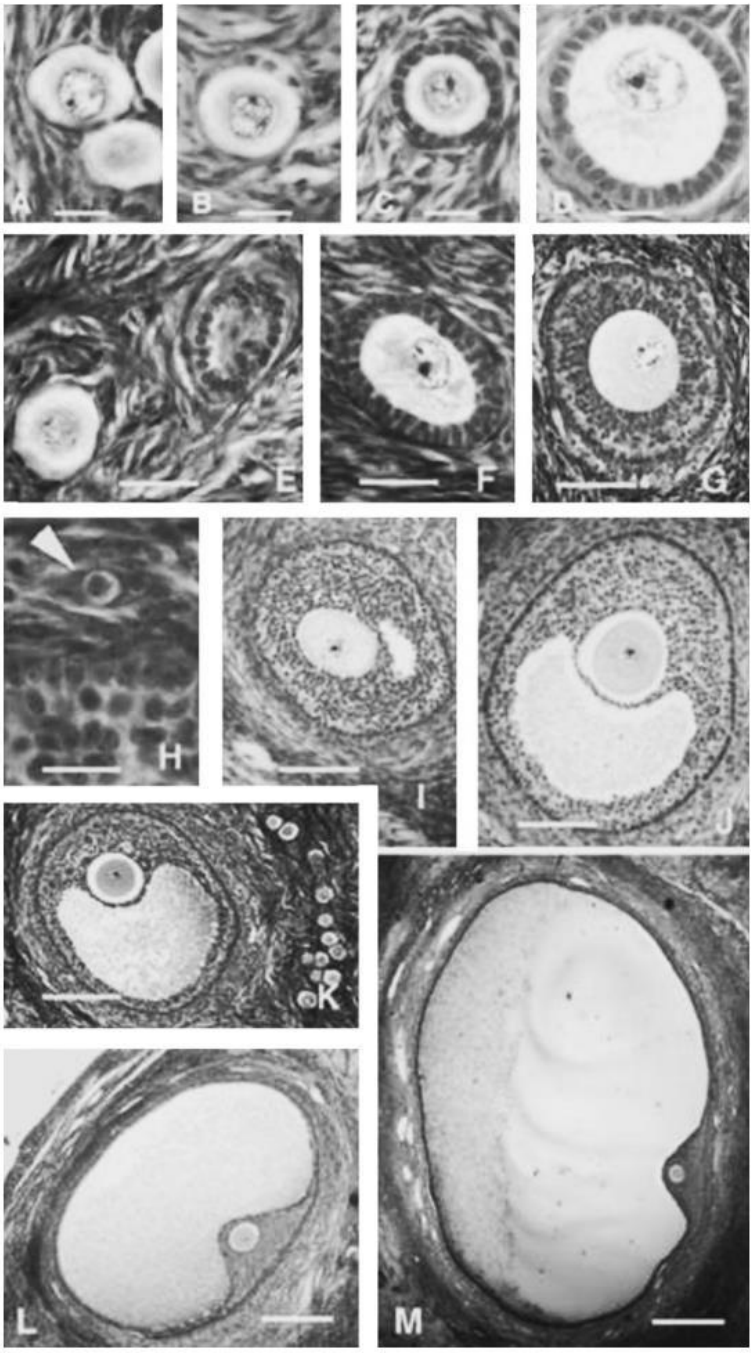

Figure 1.1 Development of human ovarian follicles. A. Primordial follicle, the oocyte is surrounded by flattened GCs (scale bar = 18μm). B. Intermediary follicle, the oocyte is surrounded by a mixture of flattened and cuboidal GCs (scale bar = 18μm). C. Small primary follicle, the small oocyte is surrounded by a single layer of cuboidal GCs (scale bar = 18μm). D. Large primary follicle in which a large oocyte is surrounded by a single layer of cuboidal GCs (scale bar = 20μm). E. Atretic primary follicle (top right) in which the oocyte has disappeared; a normal primordial follicle (left) is present (scale bar = 30μm). F. Secondary follicle (scale bar = 40μm). G. Preantral follicle (class 1, 0.15–0.2 mm) (scale bar = 75m). H. High power micrograph of epithelioid theca cells (white arrow) from a preantral follicle (scale bar = 18m). I. Follicle with a small antrum (class 2, 0.2–0.4 mm) (scale bar = 100m). J. Early antral follicle; the GCs surrounding the oocyte constitute the cumulus

oophorus (class 2) (scale bar = 90m). K. Small antral follicle (class 3, 0.5–0.9 mm); resting follicles are on right (scale bar = 160m). L. Small antral follicle (class 4, 1–2 mm) (scale bar = 300m). M. Selectable follicle (class 5, 2–5 mm) (scale bar = 530m) (Gougeon, 2010).

1.1.2 Ovarian stroma

Ovary mainly consists of two components: (1) the parenchyma, which is the specialized tissue responsible for the organ's function, and (2) the stroma, which serves as the supportive tissue (Garcia *et al.*, 2018). The ovarian follicles, functioning as the primary units of the ovary, constitute the ovarian parenchyma (Aidagulova *et al.*, 2007). However, the ovarian stroma refers to the components that are distinct from ovarian follicles, which mainly contain general elements, such as immune cells, blood vessels, nerves, and lymphatic vessels, as well as the unique ovarian components (Wu *et al.*, 2004; Meirow *et al.*, 2007; Brown and Russell, 2014; Li *et al.*, 2022). These unique ovarian components include but are not limited to the ovarian surface epithelium, tunica albuginea, intraovarian rete ovary, hilar cells, ovarian stem cells, a variety of incompletely characterized stromal cells such as fibroblast-like, spindle-shaped, and interstitial cells, and potentially other cell types (Hanna and Hennebold, 2014; Li *et al.*, 2021b). Apart from these cellular components, the ovarian extracellular matrix (ECM) plays a crucial role in providing structural and functional support for the surrounding cells and represents an indispensable component of the stroma (Figure 1.2) (Berkholtz *et al.*, 2006; Kinnear *et al.*, 2020).

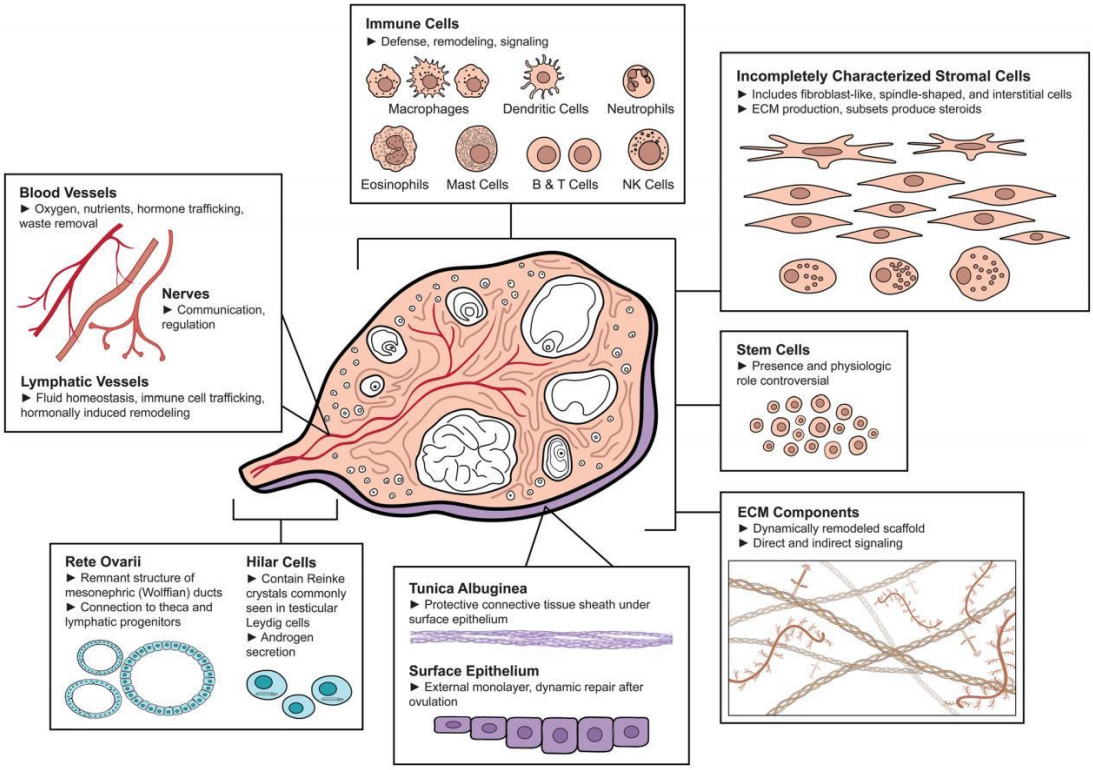

Figure 1.2 The components of the ovarian stroma. Central diagram of a human ovary surrounded by boxes highlighting different ovarian stromal components including (clockwise from top centre): immune cells including macrophages, dendritic cells, neutrophils, eosinophils, mast cells, B & T cells, and Natural Killer cells; incompletely characterized stromal cells (including fibroblast-like, spindle-shaped, and interstitial cells); stem cells; ECM components; surface epithelium and tunica albuginea; rete ovary and hilar cells; and blood vessels, lymphatic vessels, and nerves (Kinnear *et al.*, 2020).

1.2 Manifestations of premature ovarian insufficiency (POI)

Premature ovarian failure (POF) is diagnosed based on three criteria: elevated follicle-stimulating hormone (FSH) levels (> 40 IU/L), four or more months of secondary amenorrhea, and age younger than 40 years (Vincent *et al.*, 2020). Around 2007–2008, the term POI was proposed to describe this condition associated with early ovarian aging. Readers of scientific literature may encounter both POI and POF, sometimes with similar or slightly varied definitions (Vander *et al.*, 2018). POI is considered more appropriate to describe this condition of premature ovarian aging, as women with POI may sometimes experience spontaneous follicular development,

resume menses, or even conceive after diagnosis. Biochemically, POI is characterized by serum FSH concentrations >25 mIU/mL and oestradiol <50 pmol/L in women who have not menstruated for 12 consecutive months (Pellicer *et al.*, 2023). POI is mainly caused by chromosomal/genetic anomalies such as Turner syndrome and fragile X syndrome, as well as from associations with autoimmune disorders, infections, or environmental factors (Kinnear *et al.*, 2020; Yang *et al.*, 2021). Simultaneously, POI can also be triggered by iatrogenic treatments, resulting from medical interventions such as surgery, chemotherapy, and radiotherapy (Dolmans and Manavella, 2019; Spears *et al.*, 2019). Generally, POI represents a clinical syndrome characterized by ovarian dysfunction occurring before the age of 40 and is primarily distinguished by a notable reduction in the number of ovarian follicles and a deterioration in the quality of eggs (Rebar and Keator, 2021). As we know, the number of follicles and eggs existing in the ovary remains constant from birth, but with ageing, various factors such as cyclic ovulation, follicle atresia, and apoptosis lead to a substantial reduction in the follicle count (May-Panloup *et al.*, 2016; Ata *et al.*, 2019). Moreover, the accumulation of metabolic byproducts in the body can modify the ovarian microenvironment, including oxidative free radicals and advanced glycation end products (Ahmed *et al.*, 2020; Chiang *et al.*, 2023). These alterations can induce deoxyribonucleic acid (DNA) damage in the follicles, resulting in telomere shortening, follicle atresia, and apoptosis.

1.2.1 The decrease in follicle number

The primitive follicles in the human ovary are fully formed before birth, and all the primitive follicles are referred to as the "follicle (storage) pool" (Kline *et al.*, 2011). Generally, follicle development involves several stages, including primitive follicles, primary follicles, pre-antral follicles, and antral follicles, before finally

developing into a mature follicle (Skinner, 2005). A mature follicle consists of a secondary oocyte and multiple layers of surrounding GCs (Gershon and Dekel, 2020). During the early stages of follicle development, particularly in the primitive follicle stage, there is only a single layer of GCs enveloping the oocyte, and the interaction between the oocyte and GCs promotes oocyte development (Grosbois *et al.*, 2020).

During early foetal development, the number of primitive follicles in the follicle pool ranges from 6 to 7 million. However, accompanied with the development and changes in the external environment, these factors can lead to the closure and apoptosis of most follicles, resulting in a sharp decrease in the total number of follicles in the ovary (Gershon and Dekel, 2020). Therefore, on the day of birth, there are only about 30,000 to 40,000 surviving follicles in the ovary, and these surviving follicles remain arrested in the prophase of meiosis until puberty (Johnson *et al.*, 2022; Lawley and Johnson, 2023). Under the stimulation of oestrogen and progesterone in puberty, the follicles regain their ability to undergo meiosis, develop into mature follicles, and complete periodic ovulation (Banerjee *et al.*, 2014). During the prolonged dormant phase of oocyte development, internal environmental pressures can cause the closure and apoptosis of a large number of primitive follicles, leading to a dramatic decline in the number of follicles, and further directly reducing female fertility (Wang *et al.*, 2022). Thus, the continuous depletion of follicles and oocytes is an important factor in ovarian ageing.

1.2.2 The decline of follicles quality

The decline in follicles quality is mainly manifested as mitochondrial DNA mutations, shortened telomeres, spindle dynamic defects, chromosomal misalignment, and non-disjunction of whole chromosomes (Faddy, 2000; Wang *et al.*, 2011). During reproductive ageing, oocytes undergo meiotic division, resulting in

chromosomal misalignment and unequal segregation, which in turn leads to newborns with chromosomal aneuploidies, such as Down syndrome and Turner syndrome (Dey, 2004). The risk of chromosomal aneuploidy in newborns is increased with maternal age increase, and oocyte chromosomal aneuploidy can also result in a decline in female reproductive capacity. As individuals age, their oocytes also age. Older oocytes are more prone to chromosomal abnormalities, which elevate the risk of foetal anomalies or genetic disorders. For instance, the incidence of Down syndrome (trisomy 21) is higher in pregnancies involving older mothers (age > 35). Until now, numerous risk factors influencing chromosome alignment and oocyte quality have been reported. For instance, the dynamics and stability of the centrosome and microtubule cytoskeleton affect the spindle integrity, playing a critical role in spindle formation and maintenance, and further, the dysfunction in these elements is likely to result in unequal chromosome segregation and subsequent aneuploidy (Zhou *et al.*, 2012; Fu *et al.*, 2014). Secondly, telomerase serves as a crucial determinant in the preservation of telomere length, and its activity in the ovary exhibits a declining tendency with female ageing, and notably, the telomerase activity in ovarian tissue is considerably higher among women under 38 years old in comparison to those aged 38 and above (Kinugawa *et al.*, 2000). In POI patients, the GCs located in ovarian follicles are demonstrated with diminished telomere length and telomerase activity when compared to that in healthy populations (Butts *et al.*, 2009).

GCs participate in the process of follicle growth and development, and they are involved in the regulation of follicle and oocyte development through hormone secretion and gap junction-mediated intercellular communication (Zhang *et al.*, 2018). Therefore, there is a positive correlation between the viability of GCs and the

quality of follicles and oocytes. It is worth noting that the GCs in follicles of adult macaque possess the characteristics of adult stem cells, but the telomeres of GCs are also shortened with increase in age (Yuan *et al.*, 2013). Consequently, measuring the length of telomeres in the peripheral GCs of oocytes can serve as an indicator for evaluating oocyte quality.

Moreover, mitochondrial dysfunction is closely related to ovary ageing (Yang *et al.*, 2020e), which mainly results from two factors. Firstly, ageing oocytes experience a significant decline in the number of mitochondria and the total amount of mitochondrial DNA (mtDNA), seriously affecting the normal development of oocyte/embryo (Murakoshi *et al.*, 2013; Simsek-Duran *et al.*, 2013). Secondly, the impaired antioxidative capacity leads to the accumulation of reactive oxygen species (ROS) and a notable increase in the mtDNA mutation rate in oocytes (Chankitisakul *et al.*, 2013). The above-mentioned two factors directly affect the quality of oocytes. Consistently, ageing oocytes experience reductions of both ATP production and metabolic activity, resulting in the disorder of crucial issues in normal reproduction, such as spindle assembly during meiosis, cell cycle regulation, chromosome segregation, embryo development, and successful implantation (Van Blerkom, 2004; Eichenlaub-Ritter, 2012). Simultaneously, GCs located in the follicle play a crucial role in the early stages of oogenesis and contain numerous mitochondria, which suffer from high levels of mtDNA deletion and mitochondrial dysfunction during reproductive ageing (Seifer *et al.*, 2002; Tatone *et al.*, 2006). Then, the mitochondrial dysfunction disrupts the communication between GCs and oocytes, leading to impaired oocyte maturation. Additionally, recent studies have demonstrated that mtDNA mutations exacerbate female reproductive ageing by interfering with the NADH/NAD+ reDox balance (Yang *et al.*, 2020b).

Indeed, the ovarian microenvironment is composed of different cell types, and the communication between oocytes and the ovarian microenvironment is mediated through direct contact with surrounding cells, ECM, and signalling molecules (including hormones, growth factors, and metabolic products) (Ahmed *et al.*, 2020). Therefore, the ovarian microenvironment can affect the quality of oocytes and even accelerate oocyte ageing, leading to infertility and other reproductive diseases. Bidirectional communication between oocytes and their surrounding somatic cells plays a crucial role in reproduction and embryogenesis, where cumulus cells can provide essential nutrients for oocyte maturation through various paracrine signalling pathways. The published report has indicated that cumulus cells are involved in regulating oocyte ageing (May-Panloup *et al.*, 2016).

Mutations and reduced expression of key genes involved in DNA repair and mitochondrial functions could accelerate follicle depletion in patients with POI (Trifunovic *et al.*, 2004). In mouse oocytes, age-associated alterations in the expression of genes involved in mitochondrial functions and oxidative stress have been observed (Hammond *et al.*, 2016). Mutations in (Polymerase Gamma 1 (POLG1) lead to increased mitochondrial DNA mutations and premature aging in mice. Additionally, patients with POLG1 mutations exhibit premature menopause phenotypes, and single nucleotide polymorphisms (SNPs) linked to the POLG1 gene have been associated with the age at natural menopause according to genome-wide association studies (GWAS) (Luoma *et al.*, 2004; Day *et al.*, 2015). Data from high-quality association studies suggest that meiosis and DNA repair genes may be responsible for 37% of POI cases (Yang *et al.*, 2021). A group of transcription factors are expressed in granulosa cells, with mutations in their genes leading to decline of follicle quality. The Forkhead box O (FOXO) genes are part of the

forkhead family of transcription factors. Mutations in FOXL2 (Forkhead box L2) have been identified in syndromic POI patients with blepharophimosis-ptosis-epicanthus-inversus syndrome (BPES) (Beysen *et al.*, 2008). Additionally, FOXL2-null mice exhibit defects in granulosa cell differentiation and infertility. Mutations in FOXL2 have also been found in non-syndromic POI patients (Uhlenhaut *et al.*, 2009; Wang *et al.*, 2010). Notably, FOXL2 is essential for preventing the trans-differentiation of an adult ovary into a testis, as inducible deletion of FOXL2 in ovarian follicles in mice leads to upregulation of testis-specific genes, including the critical SRY (Sex determining region Y) target gene SOX9 (SRY-box 9) (Watkins *et al.*, 2006). Furthermore, missense mutations in another FOXO family gene, FOXO3, which is primarily expressed in oocytes, have been detected in POI patients (Gallardo *et al.*, 2008). This finding is consistent with studies demonstrating the essential role of FOXO3, downstream of the Akt signalling pathway, in controlling the initiation of primordial follicle growth (Ting and Zelinski, 2017).

1.3 The impact of POI on other systems

1.3.1 The impact of POI on urinary and reproductive systems

The decrease in oestrogen levels caused by ovarian ageing is associated with Genitourinary Syndrome of Menopause (GSM) (Cox *et al.*, 2023). Oestrogen receptor (ER) in the urinary and reproductive tract are highly sensitive to oestrogen deficiency caused by ovarian ageing (Calleja-Agius and Brincat, 2015). The deficiency of oestrogen leads to the degradation of collagen, elastin, and smooth muscle in connective tissue, resulting in a reduction of epithelial cells, thinning of tissues, diminished blood flow, and alterations in the microenvironment (Craciunas *et al.*, 2022). Generally, GSM includes symptoms and signs such as genital atrophy,

urinary tract atrophy, and sexual dysfunction (Pérez-López *et al.*, 2021). And the most common manifestations are vaginal dryness, itching, recurrent urinary tract infections, decreased libido, and difficulties with sexual intercourse (Faubion *et al.*, 2017). Moreover, GSM usually occurs after ovarian function declines and gradually exacerbates. Over 50% of postmenopausal women suffer from GSM, while it is even more prevalent among cancer patients, significantly affecting the life quality of perimenopausal and elderly women (Gandhi *et al.*, 2016).

1.3.2 The impact of POI on skeletal and musculoskeletal systems

The impact of ovarian ageing on women's bone health is definite (Li and Wang, 2018; Nash *et al.*, 2022). Osteoclasts are direct targets of oestrogen action, and oestrogen can induce apoptosis in osteoclasts, reducing bone resorption (Lupsa and Insogna, 2015). Simultaneously, oestrogen can also promote the secretion of calcitonin, indirectly inhibiting osteoclast viability, as well as acting on osteoblasts to inhibit the apoptosis of osteoblasts and osteoclasts (McNamara, 2021). Additionally, decreased oestrogen levels lead to improved osteoclast viability, accelerating age-related bone loss, and are an important risk factor for postmenopausal osteoporosis, and early menopause increases the risk of osteoporosis by 1.83 times and fragility fractures by 1.68 times (Svejme *et al.*, 2012).

1.3.3 The impact of POI on the cardiovascular system

Cardiovascular disease (CVD) is the leading cause of death in women, and oestrogen is recognized as a protective factor for CVD due to its effects on preserving vascular endothelium, inhibiting vascular calcification, regulating vascular function, and combating atherosclerosis (Bayoumi and Karasik, 2021). Compared to age-matched men, premenopausal women have a lower incidence of CVD, but this difference disappears after menopause (Shufelt *et al.*, 2018). Early

menopause has been repeatedly identified as a risk factor for CVD and related mortality rates (Bucciarelli *et al.*, 2020). Compared to women with a natural menopause age of 50-54 years, the risk of CVD is gradually increased in women with a natural menopause age of 45-49 years and 40-44 years (Price *et al.*, 2021). Simultaneously, premature ovarian failure and surgical menopause resulting from bilateral oophorectomy increase the relative risk of CVD by 2.2 and 8.7 times, respectively (Lokkegaard *et al.*, 2006). Additionally, the anti-Müllerian hormone (AMH) levels in the whole reproductive cycle can be taken as an independent predictor of CVD risk during a 20-year follow-up. For every 1 ng/ml decrease in logAMH, the risk of CVD increases by 21%, and the risk of coronary heart disease increases by 26% (Nelson *et al.*, 2023).

1.3.4 The impact of POI on mental and nervous systems

During the process of ovarian ageing, women may experience a series of clinical symptoms, including anxiety, depression, cognitive impairment, and sleep disorders, which are likely to result from the deficiency of oestrogen caused by ovarian ageing (Rivera *et al.*, 2009). The ER network is one of the major regulatory factors in the nervous system, and the insufficiency of oestrogen can lead to dysfunctions in signal transmission, neural circuit function, and energy metabolism within neural cells, thereby resulting in related mental and psychological symptoms (Sochocka *et al.*, 2023).

Although anxiety exhibits a greater impact on women with ovarian ageing and is more likely to be noticed, the occurrence of depression is more common (Woods *et al.*, 2023). A prospective community cohort study showed that the incidence of anxiety symptoms in premenopausal women was 3.1%, which significantly increased to 7.0% and 7.4% in the perimenopausal and postmenopausal

women, respectively. Meanwhile, the prevalence of depressive symptoms increased from 14.5% in the premenopausal women to 18.2% and 19.6% in the perimenopausal and postmenopausal women, respectively (Tang *et al.*, 2019). As oestrogen levels decline during the process of ovarian ageing, women may experience significant cognitive decline, particularly in learning and memorial functions. It is also known that oestrogen plays a role in increasing neurotransmitter levels, enhancing synaptic plasticity, and regulating signal transmission (MacLennan *et al.*, 2006).

Sleep disorders could occur throughout the whole life cycle, and their occurrence or severity tends to increase during the progression of ovarian ageing. The prevalence of difficulty in falling asleep increased from 37.5% in premenopausal women to 65.95% in postmenopausal women, while nighttime awakening increased from 28.12% in premenopausal women to 50.29% in perimenopausal women, and further increased to 53.71% in the postmenopausal women (Luo *et al.*, 2020). Although the exact mechanisms are not fully understood, anxiety, depression, and increased follicle-stimulating hormone (FSH) levels are independent risk factors for difficulty in falling asleep. Additionally, women with premenopausal insomnia have a higher risk of experiencing moderate to severe insomnia during the perimenopausal period (Brown and Gervais, 2020).

1.4 Commonly used chemotherapeutic agents for cancer patients

The research on anti-tumour drugs began in the 1940s, and over the past several decades, significant progress has been made through the collective efforts of scientists (Chabner and Roberts, 2005). Among hundreds and thousands of synthetic compounds and extracts from natural products, over 1,000 compounds with effective antitumour activity in animal models have been obtained, and more than 100 of them

are further confirmed to be effective in the clinic, partially satisfying the therapeutic requirements of some types of tumours (Mehrling, 2015). Among these anti-tumour drugs, only a few were discovered in the 1940s, primarily including alkylating agents such as nitrogen mustard and antimetabolites such as methotrexate (Karati *et al.*, 2022). In the 1950s, alkylating agents made significant advancements, while antitumour antibiotics such as streptozocin and the plant-derived anticancer drug vincristine also successively explored due to the fast development in anti-tumour research field (DeVita and Chu, 2008). Subsequently, in the 1960s to 1970s, alkylating agents, such as carmustine, were developed, which were capable of crossing the blood-brain barrier and used for brain tumours treatment. Furthermore, antibiotics such as bleomycin and Dox, exhibiting distinct characteristics and good therapeutic effects, were also developed (Falzone *et al.*, 2018). Currently, six primary categories of chemotherapeutic drugs are widely utilized.

1. Anthracycline: Anthracyclines are compounds derived from substances produced by the fungi *Streptococcus peucetius* or *S. caesius* (idarubicin being a synthetic derivative). This group of drugs mainly includes daunorubicin, idarubicin, and Doxorubicin (Dox), as well as newly-developed analogues such as valrubicin, epirubicin, and mitoxantrone, which play critical roles as antitumour agents. Generally, anthracycline impedes the transcription of mRNA and hinders the synthesis of DNA and RNA through intricate mechanisms. Exemplified by Dox, are broad-spectrum anti-tumour drugs which specifically attach to DNA and topoisomerase II, leading to the inhibition of DNA replication (Chang *et al.*, 2023).

2. Antimetabolite: Antimetabolites are a class of drugs that undergo cellular metabolism to generate structural analogues of metabolic intermediates, which competitively inhibit key enzymes involved in the biosynthesis of folate, pyrimidines,

and purines (Mease *et al.*, 2024). Methotrexate, fluorouracil, and cytarabine are the representative drugs in this category. Methotrexate, an antifolate, played a pivotal role in achieving the first remission of leukaemia and successfully treating the first solid tumour (choriocarcinoma) (Puig, 2014). The main mechanism of the methotrexate-induced antitumour effect involves inhibiting the activity of dihydrofolate reductase, which is responsible for converting folate into tetrahydrofolate (FH4), and through inhibiting the enzyme activity, methotrexate causes a significant accumulation of FH4 polyglutamate, a toxic inhibitory substrate for tumour growth (Kanarek *et al.*, 2018).

3. Antimitotic agents: The normal process of cellular mitosis requires two crucial mechanisms: the polymerization and depolymerization of microtubules within the mitotic spindle. Several anti-mitotic drugs, primarily derived from natural plant alkaloids such as vincristine and paclitaxel (Tax), can exert their therapeutic effects by perturbing the intricate dynamics of tubulin polymerization and depolymerization (Sati *et al.*, 2024).

4. Topoisomerase inhibitors: Topoisomerases play critical roles in maintaining the topological structure of supercoiled DNA double helices. Inhibition of topoisomerase I can impede the process of DNA strand cleavage, resulting in perturbed DNA replication. Similarly, topoisomerase II inhibitors form complexes with both the enzyme and DNA, thereby disturbing the rejoining reaction of fragmented DNA (Talukdar *et al.*, 2022). Until now, the typical representatives belonging to this class include irinotecan, etoposide, and teniposide (Delgado *et al.*, 2018).

5. Platinum-based agents: Mechanisms of platinum-based agents-induced antitumour activity include formation of platinum-DNA adducts, disruption of DNA

replication, , and arrest cell cycle (especially in G2/M phase) (Yu *et al.*, 2022). Prominent examples of these drugs include cisplatin (Cis), carboplatin, and oxaliplatin (Makovec, 2019). Cis, a pioneer among first-generation platinum complexes, is served as a frontline treatment for various solid tumours, establishing its position as one of the most extensively applied and potent anticancer agents (Ghosh, 2019). Generally, Cis penetrates the cell membrane via diffusion through equation, and it undergoes a transformation where a chloride ligand is displaced by water molecules, resulting in the formation of an active compound (Hanif and Hartinger, 2018). Furthermore, the chemical displacement enables the platinum atom to crosslink with DNA, such as guanine, thereby creating polymers that disrupt the normal progression of cell division (Lazarević *et al.*, 2017).

6. Alkylating agents: These agents possess active alkyl groups that establish covalent bonds with macromolecules, particularly DNA in the nucleus. Consequently, they induce damage to the DNA template, impede DNA replication and transcription, and exhibit no specificity for the cells staying in any phase of the cell cycle. The representatives of alkylating agents include nitrogen mustard, cyclophosphamide (CTX), ifosfamide, and dacarbazine (Chiorcea-Paquim and Oliveira-Brett, 2023).

The application of chemotherapy has greatly extended the survival time of tumour patients. The introduction of alkylator-based combinational chemotherapy protocols, such as the regimen comprising CTX, Dox, and vincristine, contributed to notable advancements in the treatment of patients with extensive-stage small-cell lung cancer (Zugazagoitia and Paz-Ares, 2022). Promisingly, the combinational regimens achieved response rates ranging from 60 to 80%, with complete response rates ranging from 15 to 25%. Simultaneously, the combinational regimens contribute to significant improvements in median survival time (Bergamini *et al.*,

2023). In the field of orthopaedic oncology, chemotherapy plays a crucial role and is widely considered the main factor contributing to enhanced survival rates (Addamiano *et al.*, 2024). A meta-analysis of 5,000 patients revealed that chemotherapy exhibited a positive correlation to the overall survival of patients with head and neck cancer (HR = 0.92[95%CI:0.85–0.99], p = 0.02) (Dauzier *et al.*, 2019). Similarly, another meta-analysis collecting data from 19,805 patients who participated in 107 randomized trials was conducted to evaluate the effects of chemotherapy on head and neck cancer. The findings demonstrated that concomitant chemotherapy significantly improved overall survival rates in patients with head and neck cancer and no distant metastasis (Lacas *et al.*, 2021). Furthermore, a retrospective analysis of cases revealed that the administration of adjuvant chemotherapy enhances the survival rates in 3–4 cm non-small-cell lung cancer patients with visceral pleural invasion (Wightman *et al.*, 2022). Two additional retrospective studies have also indicated that the utilization of adjuvant chemotherapy significantly enhances the survival of patients with non-low-grade and well-differentiated appendiceal cancer, as well as older patients diagnosed with metastatic pancreatic cancer (Jain *et al.*, 2020; Kolla *et al.*, 2020).

However, even though chemotherapy can prolong the survival time of tumour patients, its side effects cannot be ignored. Existing research findings indicate that chemotherapy can cause serious cardiotoxicity, peripheral neuropathy, nephrotoxicity, gastrointestinal toxicity, ovarian toxicity, and so on (Zraik and Heß-Busch, 2021).

1.5 Side effects of chemotherapeutic agents

Globally, there is an increasing and age-related trend in the incidence of malignant tumours. In recent years, benefiting from the advancements in tumour surveillance and diagnostic methods, there has been a significant improvement in the survival rates of cancer patients (Rahimzadeh *et al.*, 2016). Chemotherapy stands as a widely employed and effective therapeutic approach for malignancies, leading to evident prognostic enhancements in numerous cancer patients. However, although chemotherapy effectively targets tumour cells, they unavoidably induce damage to other normal organs and tissues, such as the heart, kidney, peripheral nerve, cochlea, gastrointestinal tract and ovary (Emery *et al.*, 2022).

1.5.1 Cardiotoxicity

Cardiovascular disease and heart failure, the prevalent late consequences of chemotherapy, undermine both the long-term survival and life quality of patients who have overcome sarcoma (Christidi and Brunham, 2021). Anthracyclines, alkylating agents, 5-fluorouracil, and Tax are frequently used as chemotherapeutic medications that possess cardiotoxicity potential, leading to the occurrences of cardiac diseases such as heart failure, coronary artery disease, hypertension, and thrombosis, and the incidence ranges from 2.3% to 48% depending on the different chemotherapeutic drugs (Curigliano *et al.*, 2016). In particular, anthracyclines exhibit high potential and in notable instances, they can intercalate within the DNA or ribonucleic acid (RNA) base pairs, effectively impeding the synthesis of these nucleic acids (Kitakata *et al.*, 2022). Additionally, anthracyclines also can exert inhibitory effects on topoisomerase IIα, a crucial enzyme involved in DNA transcription and replication. Furthermore, anthracyclines induce oxidative stress mediated by iron, resulting in DNA, protein, and lipid damage, as well as histone

modification that disrupts epigenomic and transcriptomic responses (Chen *et al.*, 2022b). Generally, anthracyclines (including Dox) induced cardiotoxicity represents a severe and frequently fatal side effect after effective tumour therapy, leading to substantial impairment in the life quality of patients. The acute injury inflicted on the juvenile heart by Dox suggests that the adult heart is increasingly susceptible to subsequent stressors, thereby elevating the risk of ischemic injury and predisposing patients to the development of cardiomyopathies at an earlier stage in life (Rawat *et al.*, 2021). Consequently, a minor ischemic incident that would typically have minimal or negligible consequences in a healthy individual, may induce more pronounced damage in the heart of patients previously impacted by DOX. Based on the estimations, approximately 60% of paediatric patients undergo a treatment regimen that includes anthracycline, and it is anticipated that within a period of up to 15 years after completing the therapy, approximately 10% of these patients will develop into symptomatic cardiomyopathy (Gianni *et al.*, 2008). Given the potential manifestation of cardiotoxicity induced by Dox, even after treatment cessation for several years, it is imperative to develop an effective intervention that can alleviate cardiotoxicity without compromising the antitumour efficacy of drugs.

1.5.2 Nephrotoxicity

Approximately 80% of patients undergoing chemotherapy are administered drugs that have the potential to cause kidney damage, considering the crucial role of kidneys in eliminating chemotherapeutic drugs (Nicolaysen, 2020). Nephrotoxicity, as a persistent and significant complications seriously impede the efficacy of cancer treatment and has a negative impact on both mortality rates and hospital stays for cancer patients receiving conventional chemotherapeutic agents or targeted therapies (Malyszko *et al.*, 2017). It is reported that for patients undergoing chemotherapy, the