Role of non-cardiomyocytes in anticancer drug-induced cardiotoxicity: A systematic review

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SUMMARY
Cardiotoxicity induced by anticancer drugs interferes with the continuation of optimal treatment, inducing life-threatening risks or leading to long-term morbidity. The heart is a complex pluricellular organ comprised of cardiomyocytes and non-cardiomyocytes. Although the study of these cell populations has been often focusing on cardiomyocytes, the contributions of non-cardiomyocytes to development and disease are increasingly being appreciated as both dynamic and essential. This review summarized the role of non-cardiomyocytes in anticancer drug-induced cardiotoxicity, including the mechanism of direct damage to resident non-cardiomyocytes, cardiomyocytes injury caused by paracrine modality, myocardial inflammation induced by transient cell populations and the protective agents that focused on non-cardiomyocytes.

INTRODUCTION
Cancer remains the most common cause of death worldwide, while cancer and heart disease are the principal causes of morbidity and mortality in industrialized countries (Curigliano et al., 2016). With the significant development of cancer treatment and the aging of patients with cancer, adverse effects of anticancer drugs increase mortality (Siegel et al., 2012). Cardiotoxicity is a severe adverse effect of anticancer drugs on the heart. Therefore, it is essential issue for cardiologists and oncologists to take countermeasures (Curigliano et al., 2016). According to the National Cancer Institute, this toxicity affecting the heart includes direct effects on the myocardium and indirect effects caused by hemodynamic changes or thrombotic events. Cardiotoxic-derived phenotypes are highly variable in pathogenesis and disease severity (Gambarrelli et al., 2017a, 2017b).

The permanent cellular component of the heart includes 56% cardiomyocytes, 27% cardiac fibroblasts, 7% endothelial cells, and vascular smooth muscle cells, which account for 10% together with transient cell populations (Souders et al., 2009; Banerjee et al., 2007). Transient cell populations include lymphocytes, mast cells, and macrophages interacting with permanent cells to affect heart function (Souders et al., 2009). Cardiomyocytes are responsible for generating electrical conduction and contractility; other cells are involved in matrix deposition, angiogenesis, inflammation, and autonomic regulation (Kofron and Mende, 2017). These cardiomyocytes are functionally correlated and work together to guarantee proper cardiac output. Although previous studies have primarily focused on cardiomyocytes, their pathogenesis has traditionally been attributed to DNA damage through topoisomerase 2β and oxidative stress (Li et al., 2021). The proposed mechanism does not wholly explain the cardiotoxicity caused by anti-tumor drugs, which illustrates the complexity of the pathogenesis. It is not hard to conclude that the cardiotoxicity caused by anti-tumor drugs is closely related to the entire heart microenvironment. In addition to the indispensable role of non-cardiomyocytes in the heart, cell-to-cell interactions can induce cardiomyocyte damage and cardiotoxicity. Therefore, it is imperative to study the role of non-cardiomyocytes in cardiotoxicity.

In this review, we summarize the role of non-cardiomyocytes in cardiotoxicity caused by antineoplastic drugs and how signal crosstalk between non-cardiomyocytes and cardiomyocytes affects the structure, contractility, rhythm, and other functions of the heart. At the end of each section, the existing studies of cardioprotective drugs that are anti-apoptotic, antioxidant, anti-fibrotic, and maintain vascular function are listed.

CARDIAC FIBROBLAST
Fibroblasts account for 40% of non-cardiomyocytes, which synthesize extracellular matrix and collagen, produce the structural framework tissue, and play a key role in promoting wound healing (Tijssen et al., 2017). The extracellular matrix produced by fibroblasts is an essential component of the myocardial extracellular matrix, which is involved in heart development, repair and remodeling. The fibroblast extracellular matrix is a dynamic structure that plays a crucial role in maintaining the integrity and function of the myocardium. The extracellular matrix consists of various components, including collagen, glycosaminoglycans, and non-collagenous proteins, which are synthesized and secreted by fibroblasts. The synthesis and degradation of the extracellular matrix are tightly regulated by various signaling pathways, including the TGF-β, Wnt, and Notch pathways. The extracellular matrix synthesis is necessary for the growth and differentiation of cardiomyocytes, while its degradation plays a role in the repair and remodeling of the myocardium. Therefore, the regulation of extracellular matrix synthesis and degradation is crucial for maintaining the normal function of the heart.

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Studies in vivo have shown that the apoptotic cell population of cardiac fibroblasts accounts for 80% of total apoptotic cells, while cardiomyocytes account for only 20% (Zhan et al., 2016). Anti-tumor drugs lead to imbalances in cardiac fibroblast function, thus inducing many heart diseases. Current molecular regulatory mechanisms of fibroblast revolve around fibroblast apoptosis and cardiac fibrosis (see Figure 1).

Cardiac fibrosis, where extracellular stroma (ECM) proteins over-clustered in the myocardial interstitium and perivascular vessels, is a marker of maladaptive hypertrophy and heart failure. It is associated with the destruction of typical myocardial structures and increased mechanical hardness, leading to cardiac contractile dysfunction (Creemers and Pinto, 2011). Moreover, Fibrosis will disrupt electrical continuity between cardiomyocytes, causing slowing of conduction and promoting the onset of arrhythmias (Yue et al., 2011). The specific mechanism of cardiac fibrosis has not yet been elucidated, and the existing research can be analyzed from the following three perspectives.

**Direct damage to cardiac fibroblasts**

Anthracyclines may cause patients to undergo early cardiac fibrosis, an essential mechanism for impaired left heart function. The cytoplasmic matrix is distributed between parenchymal cells such as cardiomyocytes and cardiac fibroblasts and is crucial in maintaining stability and repairing damaged cells. Moreover,
Dox directly affects cardiac fibroblasts, resulting in a dose-dependent increase in left ventricular collagen in the heart (Levick et al., 2019). When the cumulative dose of Dox has not yet reached the toxic dose, the direct effect of Dox on Cardiac fibroblasts is reflected in "reactive fibrosis" rather than "alternative fibrosis" (Tanaka et al., 2020). Once the cells are subjected to more significant damage, such as myocardial infarction, the regenerative capacity of peripheral cells is exceeded. Cardiac fibroblasts will induce the damaged cell to shed parts, replace the necrotic cells, form a scar dominated by type I collagen, and then mechanically fix the tissue defect area. Conversely, Cardiac fibroblasts persistently activated by chronic stress and inflammation (such as hypertension and diabetes) can induce reactive fibrosis (Russo and Frangogiannis, 2016). Reactive fibrosis leads to increased ECM deposition without significant cell loss. Initially, reactive fibrosis occurs around the blood vessels and spreads to the interstitial zone (Tanaka et al., 2020). Reactive fibrosis initially manifests as a decrease in mitochondrial membrane potential accompanied by the induction of an increase in autophagy to remove damaged mitochondria.

Endogenous substances that cause a sterile inflammatory response are called damage-associated molecular patterns (DAMPs). Damaged mitochondria are well known as DAMPs that activate the innate immune system. DAMPs derived from mitochondria include the damaged mitochondria itself, mitochondrial DNA, mitochondrial transcription factor A, and so on. When mitochondria are damaged, the release of DAMPs increases and is recognized by Toll-like receptor 9 (TLR9). The secretion of IL-1β was continuously increased. In addition, an increase in P53 can also induce mitochondrial damage in cardiac fibroblasts, resulting in a decrease in mitochondrial membrane potential (Zeisberg et al., 2007). Previous studies have shown that substance P, a neuropeptide containing 11 amino acids in the heart, promotes the development of fibrosis through the neurokinin-1 receptor (NK-1R). NK-1R is expressed in cardiomyocytes, cardiac fibroblasts, and endothelial cells, and blocking NK-1R can partially reduce cardiac fibrosis (Levick et al., 2019).

Under various stressors, Cardiac fibroblasts are transdifferentiated into myofibroblasts. Owing to the expression of α-smooth muscle actin (α-SMA), myofibroblasts synthesize more extracellular matrix and obtain contractile activity (Zeisberg and Kalluri, 2010; Kong et al., 2014). Dox act as stressors and induces the transdifferentiation of human cardiac fibroblast (HCFs) and cardiac fibrosis by upregulating inflammatory cytokines and matrix metalloproteinase 1 (MMP1) (Narikawa et al., 2019). This transformation occurs only in HCFs while no corresponding phenomenon is found in human dermal fibroblasts or mouse fibroblasts. Furthermore, cell and race specificity are present.

It is worth noting that the direct injuries of HCFs here are built based on repairing myocardial damage. Subsequent studies have been conducted in isolated HCFs and found that Dox still leads to collagen increase, i.e., Dox directly affects HCFs. However, it is interesting that the rise of collagen did not cause apoptosis of HCFs but increased their vitality, suggesting that the occurrence and development of cardiac fibrosis are not only related to alternative fibrosis and myocardial injury but may be the result of the coexistence of some mechanism (Levick et al., 2019). However, its specific mechanism has yet to be elucidated.

On the other hand, changes in the activity of critical factors caused by anticancer drugs also induce the aggregation of ROS, thereby inducing apoptosis of cardiac fibroblasts. Cardiac fibroblasts, which make up 70% of non-cardiomyocytes, synthesize ECM components and play a significant role in the development of the heart by secreting extracellular matrix components and matrix metalloproteinase (MMPs) to proliferate, migrate and reshape the cardiac interstitium (Tanaka et al., 2018). Following studies have shown that the production of cardiac fibrosis is accompanied by the activation of MMPs, because MMPs can degrade ECM in heart tissue and digest type-1 and type-3 collagens, critical extracellular matrix components in myocardial tissue (Ahmed et al., 2006). MMPs are considered key regulators of cardiac fibrosis, affecting the structural characteristics and function of the left ventricle (Tanaka et al., 2018). In particular, MMP1, MMP7 are star proteins in non-cardiomyocyte-induced cardiotoxicity. The mechanism of change in activity and the development of inhibitors to protect cardiac fibrosis have seen many related reports, which will be mentioned in different parts of the article.

In HCFs, Dox significantly increases the expression and activity of the MMP1 gene and exerts a fibrotic effect by PI3K/Akt, which can be effectively reversed with PI3K inhibitors (Narikawa et al., 2019). Protease-activated receptor-1 (PAR-1) is a G protein-coupled receptor expressed in Cardiac fibroblasts and can be activated by MMPs. Researches have shown that the activation of PAR-1 enhances Dox-induced
mitochondrial dysfunction in Cardiac fibroblasts and is associated with the apoptosis of Cardiac fibroblasts (Ghosh et al., 2016). Motility-related Protein 1 (MRP1), secreted by cardiac fibroblasts, is significantly elevated in Dox-induced cardiac fibroblasts, while the deletion of the MRP1 gene exacerbates heart damage and leads to significant cardiac dysfunction. Reduced glutathione (GSH) and oxidized glutathione (GSSG) are known as MRP1 substrates and are critical components of antioxidant damage, widely expressed in cardiac fibroblasts. The expression of GSH and GSSG in Mrp1−/− Cardiac fibroblasts was significantly increased and further increased under the induction of Dox, suggesting that MRP1 may protect Cardiac fibroblasts by regulating the extracellular redox state, but GSH/GSSC did not differ significantly in Mrp1−/− and Mrp1+/+ mice and the hypothesis was not further validated in this article (Zhang et al., 2016).

**Crosstalk between cardiac fibroblasts and cardiomyocytes**

Following the death of cardiomyocytes, a complex adaptation process begins. The resident cardiac fibroblasts try to repair structural damage to the myocardial wall and maintain cardiac output. This remodeling often becomes maladaptive when facing external stress, such as repeated chemotherapy exposures, in which cardiac fibroblasts release cytokines that further promote oxidative stress in cardiomyocytes (Schunk et al., 2013).

Cardiac fibroblasts are involved in the secretion of cytokines and growth factors in human heart tissue, which is one of their momentous functions. Many of the heart damage induced by Dox are attributed to the signaling crosstalk of myocardial fibroblasts. When cardiomyocytes are exposed to Dox, NADPH oxidase 1 (NOX1), a non-phagocytic isofrom of superoxide-producing NADPH oxidase, significantly upregulated cardiomyocyte proliferation. The degree of cardiac fibrosis weakened in mice after knocking out the NOX1 gene, and the survival rate of mice increased significantly (Iwata et al., 2018). The increase in NOX1 may be mediated by the activated TLR4 by DAMPs. It is well known that increased ROS is one of the critical mechanisms of heart damage, and how ROS produced by NOX1 leads to cardiac fibrosis has yet to be further elucidated.

Cardiac fibroblasts are the primary source of interleukin-6 (IL-6), whose levels correlate with the severity of heart failure, myocardial infarction, and cardiac fibrosis in heart tissue (Wang et al., 2016). In the early stages of Dox administration, IL-6 and transforming growth factor β (TGF-β) showed a dose-dependent increase, indicating that the inflammatory factors in HCFs were stimulated and overexpressed. Although in the late stages, collagen production was encouraged. The TGF-β/Smad pathway is a familiar signaling pathway that still mediates fibrosis and inflammation in HCFs (Tsai et al., 2019). After the administration of TGF-β/Smad inhibitors, the expression of IL-6 and α-SMA decreased, indicating that the inflammatory response and interstitial phenomenon were suppressed (Narikawa et al., 2019).

Further studies found that activating inflammatory factors TGF-β and IL-10 was associated with increased secretion of typical G Protein subunit beta 5 (Gβ5) in Ventricular cardiac fibroblasts (Chakraborti et al., 2018). One of the characteristics of cardiac fibroblasts is neighboring cardiomyocytes. In co-culture systems, TGF-β upregulation is more pronounced, and the effects of Gβ5 are amplified, suggesting the presence of Gβ5 as a signal regulator between cardiac fibroblasts and cardiomyocytes.

Ataxia telangiectasia mutated (ATM) kinase as a member of the phosphoinositide 3-kinase-related protein kinase (PIKK) family, its expression level was higher in cardiac fibroblasts (Zhan et al., 2010). Even if Akt is expressed in cardiomyocytes, specific knockout of the Akt gene in cardiomyocytes does not improve Dox-induced cardiotoxicity, while the opposite effect is achieved in cardiac fibroblasts. Following studies have found that Akt acts on Fas ligand (FasL) in cardiomyocytes by paracrine, mediating cardiomyocyte apoptosis and left heart failure, and using Akt selective inhibitor KU5593 can prevent Dox-induced cardiotoxicity (Zhan et al., 2014). The ATM/PS3 pathway is thought to mediate ROS-induced apoptosis, and Gβ5 inhibits the activation of the cascade pathway when the upstream factor was knocked out (Minotti et al., 2004).

The mechanisms of cellular interactions within the heart are complex and further studies are required to understand their suitability for involvement in cardiotoxicity. This could be the main target of next future studies which will allow in this manner the identification of new therapeutic strategies, based on the targeting of molecules that mediate cell communications.
**Protective agent that focused on cardiac fibroblasts**

Clinically, the cardiotoxicity of anti-tumor drugs has always been a thorny issue for which there is no breakthrough. Many scientists have followed suit and optimized various ways to reduce drug-induced cardiotoxicity, including changing the mode of transportation, such as transporting drugs in the form of drug precursors or liposomes, developing new medications, and developing cardio-protectors. Many medicines with protective effects have also been developed and explored from many different angles for fibrotic hearts. The view of telomere theory is that the shortening of chromosomal telomeres leads to cell senescence and blocking of cell replication. When cells are exposed to different stressors, cells age prematurely called stress aging. The former is a normal cell cycle, but the latter’s presence is often conspicuous (Campisi, 2013; Munoz-Espin and Serrano, 2014; Campisi and D’Adda, 2007). TM5541, an inhibitor of plasminogen activator inhibitor-1 (PAI-1), has the protective effect on stress-induced and aging-induced cellular senescence via upregulation of ROS quenchers, such as antioxidant catalase and suppression of senescence regulators Insulin-like growth factor binding protein 3 (IGFBP3) and p16-p21-P53-PAI-1 signaling pathway (Ghosh et al., 2016).

Rosmarinic acid (RA) is a natural polyphenol compound with a series of biological effects such as anti-inflammatory, antioxidant, and anti-tumor (Elufioye and Habtemariam, 2019). RA therapy can mitigate cardiomyocyte apoptosis and cardiac dysfunction in cardiotoxicity by targeting cardiac fibroblasts (Zhang et al., 2019). The primary mechanism of the cardioprotective drug, like the Akt inhibitor A5593, is to inhibit Fasl by paracrine. Recent studies have shown that RA-mediated nuclear factor of activated T-cells (NFAT) inactivation and inhibition of MMP7 expression play an important role in protecting cardiomyocyte apoptosis.

Liguzinediol, a compound derived from the structural modification of natural active ingredient ligustrazine, is often used for occlusive vascular disease, cerebral thrombosis, vasculitis, and coronary heart disease in the clinic (Di Emidio et al., 2009). Studies have shown that Liguzinediol can significantly reduce Dox-induced cardiac fibrosis, along with hydroxyproline, one of the main components of collagen, considerably decreasing the ratio of type I/III collagens. The primary mechanism of relieving cardiac fibrosis is to down-regulate the expression of MMP1, MMP2, and MMP7 and upregulate the expression of tissue inhibitor of metalloproteinase (TIMP) (Wu et al., 2016).

As mentioned above, the pathogenesis of reactive fibrosis is related to the recognition of DAMPs by TLR9, leading to an increase in inflammatory factors such as IL-1β and TIMP-1. Activation of these inflammatory factors is closely related to the emergence of fibrosis, which can be alleviated with ODN2088 (TLR9 antagonist). Although chloroquine can inhibit lysosomal action effectively, it has not been widely used because of its great side effect and high price. As a common drug in diabetes mellitus (type II) treatment, pioglitazone can be used to alleviate fibrosis by inhibiting SAPK/JNK activation and releasing inflammatory factors, While it is unknown whether it will affect the effect of chemotherapy drugs (Tanaka et al., 2020). Moreover, small doses of Dox were used in the article for research, whether fibrosis can be eliminated is uncertain.

**ENDOTHELIAL CELL**

Owing to the emerging role of Endothelial cells in supporting cardiomyocyte survival and function, recent studies have identified endothelium as a novel target for therapy (Luu et al., 2020). Coronary vascular endothelial cells are involved in maintaining the health and function of Cardiomyocytes. Endothelial cells form a physical barrier to protect Cardiomyocytes from circulating toxic substances and secrete cardioprotective molecules, including NO, endothelial-derived prostaglandin I2, and proteins that support cardiomyocyte function, such as NeuRegin-1 and endothelin-1 (Luu et al., 2018a, 2018b). Chemotherapy drugs induce endothelial cell apoptosis by disrupting the normal function of endothelial cells, thereby denying the protective and supporting effect of endothelial cells on cardiomyocytes, making myocardial tissue more susceptible to damage. The microstructures formed by human microvascular endothelial cells and Cardiac fibroblasts co-cultured with Cardiomyocytes have a more mature phenotype and contractile ability than the individually cultured cardiomyocyte, which has more advantages in predicting the exertion effect of drugs, indicating the crucial role of cardiac non-cardiomyocytes in mediating functional cardiotoxicity (Ravenscroft et al., 2016).

Particularly, Dox will severely affect endothelial cells and reduce the capillary density of treated mouse hearts, induce human microvascular endothelial cells barrier perturbation, reduce barrier function, increase the permeability of cardiac microvascular, and inhibit the formation of vascular networks in human heart
endothelial cells cultured in vitro (Wilkinson et al., 2016). Apoptosis is not considered the primary mechanism for Dox-induced endothelial cell damage. However, other mechanisms have been proposed, including the regenerative capacity of Endothelial cells precursor cells, abnormal activation of TGF-β pathways, and impaired autophagy function dependent on angiotensin-converting enzyme7 (Jahn et al., 2020; Sun et al., 2016). Here, we summarize from the aspect of vascular endothelial disorder, signal interaction between endothelial cells and cardiomyocytes, and anti-cardiotoxic endothelial cell protector (see Figure 2).

**Autocrine mechanism of vascular endothelial disorders**

There are two main types of endothelial cells in the heart: endocardial endothelial cells and vascular endothelial cells. Vascular endothelial cells are mainly distributed in the dense layer of the ventricular muscle.
Endothelial cells form a monolayer on the surface of the lumen of the vascular system, providing an interface that regulates the circulation and passage of solutes and exogenous substances between cells and tissues across cells to cells. Many studies have shown that vascular endothelial disorders and destruction are associated with anti-tumor anticancer drug-induced cardiotoxicity development.

Vascular endothelial growth factor (VEGF) is a specific mitogen of vascular endothelial cells, which promotes the growth of vascular endothelial cells in vitro and induces vascular hyperplasia in vivo. Especially in the hypoxic environment, VEGF binds to VEGFR on the endothelial cell membrane, causing its self-phosphorylation, thereby activating mitogen-activated protein kinase (MAPK), realizing the mitogen properties of VEGF and inducing endothelial cell proliferation. There is no shortage of literature on VEGF and MAPK in cardiotoxic studies.

VEGFR2 is a highly expressed tyrosine kinase receptor in endothelial cells, and its related signaling determines whether heart growth is physiological or pathological (Liu et al., 2020). In order to study the short-term effects of Dox, a model was established that endothelial cells are briefly exposed to low concentrations of Dox and analyzed a few days after drug clearance (Graziani et al., 2022). Studies have shown that Dox leads to the aging of Endothelial cells and continuously inhibits the expression level of VEGFR2. Although VEGFR1 and Fibroblast Growth Factor Receptor 1 (FGFR1) were similarly suppressed, they returned to initial levels a few days under drug clearance, and only VEGFR2 was permanently affected by Dox. VEGFR2 levels were halved within 6 h of Dox treatment, in that autophagy can degrade proteins in a short period according to previous research experience, though it was not a decisive factor. Reversely, Dox decreases VEGFR2 levels by inhibiting mTOR and persists after sloping administration (Graziani et al., 2022).

The survival and proliferation of endothelial cells are regulated by MAPKs. There are four main MAPKs: ERK1/2, JNK, P38, and ERK5 (Nithianandarajah-Jones et al., 2012, 2014). Extracellular regulated protein kinases (ERK) signaling is an important medium for VEGFR2 to activate angiogenesis and arterial production (Monti et al., 2013). Although Dox-induced ROS production in cardiomyocytes has become a consensus, the same conclusion cannot be derived from endothelial cells. Dox does not directly induce mitochondria activation and ROS production in cardiac vascular endothelial cells under a certain concentration, but activates PS3 by participating in p-ERK1/2-dependent signaling pathways, inducing caspase-3 lysis and apoptosis, thereby reducing cell viability. ERK1/2 has recently been described as an important regulator of PS3 phosphorylation and apoptosis in Dox-exposed cardiomyocytes. However, A contrary conclusion is that, dox significantly reduces the phosphorylation of ERK1/2 and that pretreatment with VEGF-B can reverse the reduction of p-ERK1/2, thereby achieving cardioprotection (Råsänen et al., 2016).

Although few studies have focused on ERK5, the related signal axis is essential during the development of the heart. Research shows that Statin mediated ERK5 activation results in the translocation of ERK5 to the plasma membrane and regulation of tight junction formation and decreased endothelial cell permeability drug permeability (Wilkinson et al., 2018). The effect on the ERK pathway needs to be further confirmed.

Primary cilia are present on endothelial cells of the vascular lumen and act as mechanical receptors for blood flow regulation. After endothelial cell primary cilia detect changes in blood flow, NO is produced to dilate blood vessels and regulate the expression of downstream signaling pathways in primary cilia (Ando and Yamamoto, 2013; Luu et al., 2018a, 2018b). Loss of primary cilia at endothelial cells accelerates Dox-mediated mortality and reduction in cardiac function, suggesting that ciliated signaling downstream pathways are potential targets for promoting endothelial cell support for cardiomyocyte function during Dox therapy (Luu et al., 2020).

**Vascular fibrosis and paracrine mechanisms**

Stress-induced fibroblasts may cause perivascular fibrosis, and the process predates cardiotoxicity. Endothelial cell-to-mesenchymal cell transformation (EndMT) is considered one of the sources of cardiac fibroblasts (Zeisberg et al., 2007). EndMT, like epithelial-mesenchymal transformation, is a phenotypic conversion process in which endothelial cells gradually transfer to the smooth muscle layer and lose their properties, acquiring interstitial properties that lead to endothelial dysfunction and interstitial fibrosis. Studies have shown that microvascular endothelial cell dysfunction plays a vital role in perivascular fibrosis (Pan et al., 2021).

Normal myocardial microvascular endothelial cells (CMECs) are flat, slender, and separated from smooth muscle cells through a thick basement membrane. After Dox treatment, CMECs lost the complete
basement membrane and presented endometrial migration and nuclear orientation changes, which are the ultrastructural characteristics of EndMT. At the same time, tube capacity decreased, and migration capacity increased. The dysfunction of endothelial cells suggests that it is involved in vascular fibrosis in the early stages of anti-tumor-induced cardiotoxicity. In chronic mouse models, fibrosis of capillaries around the heart occurs in the pre-administration period. In contrast, fibrosis between cardiomyocytes is not apparent until late.

ROS-induced the activation of NF-κB-Snail signaling pathway could be a possible approach to Dox-induced EndMT, with autophagy disorders responsible for ROS accumulation (Pan et al., 2021). Autophagy is a conservative pro-survival mechanism that circulates and removes damaged subcellular components. After the autophagosome matures, it binds to the late lysosome to form autophagic lysosomes and degrades the damaged components. Autophagy-related gene 7 (ATG7) is an important mediator that catalyzes several steps in the maturation of autophagosomes and is critical for the clearance of toxic protein aggregates from mitochondrial autophagy and oxidative stress. Endothelial cell-specific knockout of ATG7 exacerbates cardiac fibrosis (Luu et al., 2021).

Irisin is a novel hormone-like myocyte factor that improves autophagy disorders and EndMT by modulating uncoupling protein 2 (UCP2) and has antioxidant effects. Notably, Irisin is secreted by cardiomyocytes and protects endothelial cells with paracrine (Pan et al., 2021). Dox-induced human cardiac microvascular endothelial cells (HCMVEC)/HCF co-culture systems increase the expression of ALK4/5 ligands and TGF-β target genes. This signaling pathway may inhibit the formation and maintenance of microvascular networks by directly influencing the proliferation and migration of endothelial cells (Sun et al., 2016). This series of studies highlight the importance of paracrine endothelium for cardiomyocyte signaling.

Neuregulin-1 (NRG-1) is a cardiac-active growth factor released by endothelial cells that promotes the hypertrophic growth of adult cardiomyocytes by binding to Erb-b2 receptor tyrosine kinase 4 (ErbB4) receptors on cardiomyocytes. Coincidentally, NRG-1 plays an anti-apoptotic role through paracrine. ErbB2, like ErbB4, is present in cardiomyocyte NRG-1 receptors. However, NRG-1 does not bind directly to ErbB2 but instead performs signal crosstalk between cardiomyocytes and endothelial cells by binding to ErbB2/ErbB4 heterodimers. Although anti-ErbB2 antibodies (1 g/mL) can eliminate cardiomyocyte hypertrophic growth within 24 h, they will also wholly inhibit their anti-apoptotic effects (Jiang et al., 2019; Lemmens et al., 2006).

**Anti-cardiotoxic endothelial cell protector**
The endothelial cells are one of the main targets of cardiotoxicity caused by anti-tumor drugs. The pharmacological protection of microvascular endothelial cells can reduce myocardial toxicity and maintain vascular function so chemotherapy drugs are entirely transported to cancer cells.

An aortic ring experiment has shown that Dox can impair the vasoconstriction and contraction function of the thoracic aorta, leading to vascular endothelial dysfunction. After combined treatment with living Ginsenoside Rg3 (Rg3), the vasodilation function was significantly improved. In addition, Rg3 significantly inhibited endothelin-1 and induced endothelial nitric oxide synthase in DOX-induced endothelial cells. Current studies demonstrate that Rg3 has the effect of reducing Dox cardiotoxicity (Wang et al., 2015).

The phenomenon that Dox induces an increase in ROS levels has been elucidated in numerous pieces of literature. ROS activation may damage DNA peroxidation, leading to altered mitochondrial membrane permeability and inducing apoptosis. The ACE containing sulphydryl protects endothelial cells and reverses their apoptosis damage without affecting the anti-tumor effects of Dox. However, its protective effect is independent of the above damage mechanism but instead of the supply of H2S and cystathionine gamma lyase, a vascular key enzyme for H2S production expressed in endothelial cells (Monti et al., 2013).

**IMMUNE CELL**

**T lymphocytes**
The immune function of T lymphocytes is mainly to resist intracellular infection, cancer cells, and allogeneic cells. Among them, “cytotoxic” T lymphocytes can promote or inhibit the proliferation and immune function of B lymphocytes or T lymphocytes. Programmed cell death protein-1 (PD-1) and cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) are two critical immune checkpoints when both bind to ligands.
expressed in antigen-presenting cells (e.g., PD-L1, B7), the immune response of T cells is suppressed, thereby regulating the immune system and promoting self-tolerance (Dong et al., 2002). Pass an above-mentioned mechanism, helps prevent immune cells from attacking themselves and other normal cells and proteins. Unfortunately, to evade elimination by the host immune system, tumor cells commonly over-express the ligands of immune checkpoint receptors, bringing T cells to a state of non-responsiveness or exhaustion (Wherry and Kurachi, 2015). In other words, PD-1 is induced to express on the surface of activated T cells, forcing T cells to ignore cancer cells as one of the self-components (i.e., preventing T cells from attacking cancer cells). When using anti-PD-1 (called atezolizumab, avelumab, and durvalumab) or anti-CTLA-4 (called ipilimumab and Tremelimumab) block the function of immune checkpoints, activated T cells can re-realize the "non-self" nature of cancer cells and release their cytotoxicity (Ishida, 2020). Immune checkpoint inhibitors (ICIs) open up a new path for cancer treatment. On March 25, 2011, the U.S. FDA approved ipilimumab for treating advanced melanoma. It has to be said that with the inhibition of negative immune regulation, the "self" component will also be attacked by T cells, resulting in a series of immune-related adverse events. Compared to other organs, the incidence of myocarditis is merely about 1%, but 46% occur life-threatening adverse events. In the existing literature, the vast majority of articles studying the relationship between immune cells and cardiotoxicity focus on ICIs, so this article uses ICIs as the entry point to briefly describe the causes of immune cells and myocardial inflammation.

It is necessary to understand the expression of PD-1/PD-L1 in myocardial subpopulations first. The maximum proportion of cardiac PD-L1 is distributed in Endothelial cells as the primary mediator for immune crosstalk and lymphocyte penetration into heart tissue (Michel et al., 2022). However, no relevant expression of PD1 was found on any included subsets of heart cells, suggesting that evidence for the number of cardiac PD1 could stem from resident lymphocytes. Clinical pathology reports show that among patients treated with anti-CTLA-4 and anti-PD-1, there are many fatal cases of fulminant autoimmune-mediated myocarditis with lymphocyte penetration. Pathology in patients with myocardial inflammation detects many immune cell infiltrations, but there is a lack of research on the mechanism of adverse events. In 2019, a study found that morphology, cardiac biomarkers, and immune cell infiltration are similar to human ICI myocarditis in cynomolgus monkeys with immune checkpoint inhibitor (Ji et al., 2019). Subsequently, many studies related ICIs came to the same conclusion: myocardial infiltrates include T lymphocytes (CD3, CD4, CD8), macrophages (CD68), and B cells (CD20). All the time, immune checkpoint inhibitor-induced myocardial inflammation has not been pathologically graded, but distinguished in a study recently (Champion and Stone, 2020). A higher level of cell infiltration (>50CD3+ cells/HpF) causes serious necrosis of cardiomyocytes, whereas lower levels of CD3+ cell infiltration (<50CD3+ cells/HpF) have a slower course. The degree of CD3+ and CD8+ T cell infiltration in high-grade myocarditis is similar to the polar cell rejection of grade 2R, except that the former lymphoid tissue cell inflammation increases and the CD68/CD3 ratio increases. Nivolumab and ipilimumab exert effective antitumor efficacy, but also serious cardiotoxic effects in co-cultures of lymphocytes and tumor or cardiac cells. Both ICIs increased NLRP3, MyD88, and p65/NF-κB expression compared to untreated cells (Quagliariello et al., 2020).

Laser confocal microscopy assays the spatial distribution of cardiac T cells, indicating T cells represented by CD3+ in anti-PD-1 treated mice show a diffuse increase in the heart, with no signs of local aggregation (Michel et al., 2022). CD4+ cells are the primary drive in the process of inducing myocardial inflammation and are more pronounced infiltrating than CD8+(Tay et al., 2020). However, another study showed that cytotoxic CD8+ T cells are the critical mediators in increasing cardiac toxicity (Du et al., 2018). On the surface, the two conclusions are contradictory, but that’s not the case. In the early stages of myocardial inflammation, CD4+ cells transdifferentiate to Th1 under the induction of Tumor Necrosis Factor-γ (TNF-γ) bringing cardiac damage and activating CD8+ cells. On advanced cardiac toxicity, increased acute mortality and decreased cardiac output are secondary to myocardial inflammation and fibrosis. Meanwhile, CD8+ cells induce cardiac fibrosis and inflammation. In a word, the top ten pathway in Genetic difference analysis are connected with immune response, are highly statistically significant, and are predicted to be activated. Strikingly, each of the top 4 enriched pathways is involved in Th cell differentiation (Th1 and Th2 pathways) (Xia et al., 2020).

There is much clinical evidence that treatment with anti-PD-1 is accompanied by cardiac immune imbalances and left-sided cardiac insufficiency. Although the depletion of CD8+ can prevent the cardiotoxicity
caused by anti-PD-1, it also affects the anti-tumor effect and therefore has no clinical significance. However, blocking Tumor necrosis factor alpha (TNF-α) protected cardiac function without affecting its anti-tumor effect (Michel et al., 2022). Transforming Growth Factor β1 (TGFβ1) is the most prevalent TGFβ isoform expressed in many types of human tumors. A high-affinity fully human antibody, SRK-181, that selectively binds to latent TGFβ1 and inhibits its activation presents profound anti-tumor responses and survival benefits (Martin et al., 2020). CTLA4+/−/PD-1+/− mice exhibit pathological features of hypoglycemia and elevated serum protein, closely related to CD45+ infiltration (Wei et al., 2021).

Macrophage

It was found that macrophages are able to attain either M1 or M2 phenotype in the microenvironment. Pro-inflammatory macrophage M1 expresses inflammatory cytokines that aggravate tissue damage, whereas anti-inflammatory macrophage, M2, produces anti-inflammatory cytokines to facilitate tissue repair (Shapouri-Moghaddam et al., 2018; Horckmans et al., 2017). M1/M2 is recognized in the field as a predictive marker, determining inflammatory damage or repair. Under Dox-induced cardiac inflammation conditions, monocytes in the blood are recruited into tissues and differentiate into macrophages, thereby the M1/M2 in mouse myocardial tissue was definitely increased, and upregulated the expression of inflammatory factor such as IL-6, IL-β (Zhang et al., 2020). Continued dominance of M1 macrophages can obstruct tissue regeneration and lead to serious consequences such as ventricular septal defect, infarct rupture, acute mitral regurgitation, aneurysm formation, and heart failure. M2 macrophages reduce inflammation and support tissue regeneration (Ferrante and Leibovich, 2012). Latifolin effectively mitigates the rise of M1/M2 and the release of inflammatory factors, thereby playing a cardioprotective role (Zhang et al., 2020). Previous studies have confirmed that the percentage of PD-L1 expressed by macrophages is inversely correlated with the degree of inflammatory infiltration but concerned with the polarization of the M2 phenotype. It’s perhaps attributed to massive necrosis of cardiomyocytes, which causes monocytes in the blood to aggregate and differentiate into M2 macrophages (Champion and Stone, 2020).

Cardiotoxicity induced by ICI therapy is associated with accelerated senescence of cardiomyocytes, manifested as cell-cycle arrest and telomere shortening. In mouse cardiomyocytes and macrophages exposed to PD-1, the expression of the pro-aging gene miR-34a-5p was dramatically increased, and the addition of miR-34a-5p inhibitors could ultimately reverse the heart damage caused by aging (Xia et al., 2020). Nevertheless, PD-1 does not cause direct damage to cardiomyocytes but transfers miR-34a-5p to cardiomyocytes through macrophage-derived exosomes. Exosomes are cell-derived vesicles that contain proteins, lipids, growth factors, microRNAs and can release growth factors through exocytosis thereby regulating crosstalk between cells, and promoting inflammation, aging, and miRNA transfer processes. Embryonic stem cell-derived exosomes increased M2 macrophages and the anti-inflammatory cytokine IL-10, which ameliorated dox-induced focal variability and cardiac remodeling (Singla et al., 2019). Coincidentally, nivolumab-mediated cardiomyopathy requires a peripheral environment to investigate the interaction between T cells and cardiomyocytes. The pro-inflammatory factor TNF-γ was up-regulated followed by the increase of phosphorylated NF-κB and STAT1 in a direct-contact co-culture model given that PD-1 on T lymphocytes requires direct contact with PD-L1 on cardiomyocytes to activate T cells, but no similar phenomena were found in the Transwell co-culture model (Tay et al., 2020).

Mast cell

Innate immunomodulation activated by mast cells has been hot in research. Cardiac mast cells are involved in many biological processes, particularly tissue remodeling (Levick et al., 2011). However, the effect of Dox on myocardial mast cells is unclear, and only one article suggests that ovarian hormone deficiency significantly increases the density of cardiac mast cells, and usage of mast cell stabilizers can reduce myocardial inflammatory response, and alleviate cardiac contractile dysfunction and fibrosis (Phungphong et al., 2020). Additionally, mast cells are able to secrete a variety of cytokines that are involved in immunomodulation (TB cells, APC cell activation). Under the current research background, mast cells may be involved in immunosuppressant-induced myocarditis.

In short, immune cell infiltration, dominated by T cells and macrophages, is indispensable in treating cancer-induced myocarditis with ICLs (see Figure 3). As a hot research field, many clinical cases have been reported for discussion and reference. The fly in the ointment is that the leading causes of myocardial inflammation have not yet been elucidated, and mechanism research is still in infancy.
Sunitinib is a novel multi-targeted oral drug for treating tumors. A large number of patients treated with sunitinib develop cardiac insufficiency, such as myocardial thickening and coronary artery vasodilation. Cardiac dysfunction and microvascular lesions described above are associated with the depletion of peripheral cells covered with endothelial cells. The loss of pericyte cells led to a 2.3-fold increase in vascular permeability, consistent with the vascular bed studies (Chintalgattu et al., 2013). Notably, the deletion of pericytes is specific, with no similar phenomena observed in either skeletal muscle cells or Dox models in mice. PDGFR

Clofazimine upregulated Cluster Of Differentiation 62 Platelet (CD62P) expression, enhanced ADP, and activation of thrombin-mediated human platelet in vitro, which appears to be a novel cardiotoxic mechanism (Anderson et al., 2018). CD62P is widely recognized as a critical factor in activating endothelial cells and reacting situations of living blood platelet. If surgery is performed in vivo, these thrombotic activities of...
Clofazimine may predispose to microvascular occlusion, exacerbat- ing the high risk of related cardiovascular disease.

**DISCUSSION AND PERSPECTIVE**

As a result of heart damage, cardiotoxicity is a very complicated phenomenon. The myocardium consists of an intricate organization, in which different cell types are interconnected within a complex ECM network (Tijsen et al., 2012). Thus, each cell type can alter its functional characteristics, such as metabolic demand, oxygen consumption, migration, and secretion, to maintain the homeostasis of the tissues. So far, this phenomenon has still been rarely studied.

It is becoming increasingly clear that cardiomyocyte responses are regulated through direct and indirect communication between cardiomyocytes and non-cardiomyocytes (Tirziu et al., 2010). When cardiomyocytes contract, fibroblasts will produce the basic ingredients of the extracellular matrix to maintain normal cardiac tissue. At the same time, endothelial cells act as a vascular functional barrier to blood vessels, regulating the transport of nutrients, oxygen, and heterogeneity from circulation to the heart (Brutsaert, 2003; Porter and Turner, 2009). The administration of anti-tumor drugs not only directly damages non-cardiomyocytes and causes cardiac dysfunction, but also leads to common cardiovascular diseases such as cardiac fibrosis, perivascular fibrosis, and myocarditis through paracrine. Many of the signaling pathways in existing studies are summarized in this article to dissect the phenomena stated above, some of which play an essential role, for instance, fibroblast-derived MMPs are considered to be critical factors in cardiac fibrosis; EndMT is attributed to inducing fibrosis, which is a novel mechanism in the progression of heart failure (Eren et al., 2014). However, the interactions between these factors and the influences on other cells still remain baffled, so great significance to evaluate the contributions of individual signaling pathways and cells should be attached.

In order to improve oncological therapeutic efficacy, current cardiology research have sought how to avoid or reduce the cardiotoxicity induced by antineoplastic drugs. Almost all studies that detect Dox-induced cardiotoxicity in vitro have been conducted at a dosage of over 1μM of Dox. However, the timing of onset and threshold of cardiotoxicity remain unclear. It may be acute, occurring within 2–3 days or delayed until 10 to 15 years after chemotherapy (Kortlever et al., 2006; Ghosh and Vaughan, 2012; Ghosh et al., 2010; Ando and Yamamoto, 2013). Except for troponin, IL-6, some articles have attempted to reveal early diagnosis or potential risk biomarkers of Dox-induced cardiotoxicity, but they are not yet mature enough to be accepted by the industry (Mancilla et al., 2020). Although some biomarkers are already such as troponin, IL-6, but further research is needed to elucidate their role in cardiotoxicity (Henri et al., 2016; Klickap et al., 2005). Therefore, further investigation of molecular mechanisms involved in cardiotoxicity is needed to discover potential biomarkers and therapeutic drugs.

Various cardioprotective agents for clinical use, such as Statins, Digoxin, Enalapril, Phenylethylamine, and Edamine Tetraacetic Acid (EDTA), have been utilized to prevent Dox-induced cardiotoxicity. Nowadays, only iron-chelated EDTA derivative Dexrazoxane is thought to chelate and reduce the number of metal ions complexed with anthracyclines, thereby reducing the formation of superoxide anions, and is currently recommended as a cardioprotective agent for specific agents for specific patients (Klickap et al., 2005; van Dalen et al., 2011). This article summarizes many non-cardiomyocyte protectors, which have been promoting safer and more effective adjuvant drug development and taking further research forward. Many drugs are targeted therapies for specific non-cardiomyocytes, used in combination with antineoplastic drugs, and possibly used in smaller individual doses by combining sensitizing effects (Khalil et al., 2012; Curtin, 2012).

**Conclusion**

As an indispensable cell population in the heart, increasing studies have been conducted on. According to cell types, this article divides non-cardiomyocytes into cardiac fibroblasts, endothelial cells, immune cells, and other cell populations and summarizes the current research on signaling pathways and mechanisms (see Table 1).

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| Cell type                  | Drug/Agent                  | Related mechanism                  | Related pathway                  | Ref                                      |
|---------------------------|-----------------------------|------------------------------------|-----------------------------------|------------------------------------------|
| Cardiac fibroblast        | DOX/KU55933                 | Apoptosis                          | ATM knockout mice                 | (Zhan et al., 2016)                     |
|                           | DOX, L732138                | Myocardial Fibrosis                | Male SD rats                      | (Levick et al., 2019)                   |
|                           | Chloroquine/                | Reactive fibrosis/sterile          | Male C57BL/6J                     | (Tanaka et al., 2020)                   |
|                           | pioglitazone                | inflammation                       |                                  |                                          |
|                           | Dox                         | FMT                                | –                                 | (Narikawa et al., 2019)                 |
|                           | Dox                         | mitochondrial dysfunction           | PAR-1 \(-/-\) C57B1/6J mice       | (Ghosh et al., 2016)                    |
|                           | Dox                         | cardiac fibrosis                   | Mrp1 \(-/-\) C57BL/6 mice         | (Tsai et al., 2016)                     |
|                           | Calcitriol                  | EndMT                              | C57/B6 male mice                  | (Chakraborti et al., 2018)              |
|                           | DOX/paclitaxel/5-FU         | Fibrotic Remodeling                | Male Swiss albino mice            | (Tsai et al., 2019)                     |
|                           | TMS441                      | cellular senescence                | –                                 | (Ghosh et al., 2016)                    |
|                           | Rosmarinic acid             | Apoptosis                          | C57/B6 male mice                  | (Zhang et al., 2019)                    |
|                           | Liguzinediol                | cardiac remodeling                 | Male SD rats                      | (Wu et al., 2016)                       |
| Endotheliocyte            | Dox                         | endothelial cell defects           | EC-IFT88 \(-/-\) C57BL/6 mice     | (Luu et al., 2020)                      |
|                           | Herceptin and doxorubicin   | cardiac permeability/endothelial   | –                                 | (Wilkinson et al., 2016)                |
|                           | Dox                         | Angiogenesis                       | Male C57BL/6 mice                 | (Sun et al., 2016)                      |
|                           | Dox                         | Autophagy                          | EC-Atg7 \(-/-\) mice              | (Luu et al., 2021)                      |
|                           | Zofenoprilat                | Apoptosis                          | HUVECs                            | (Graziani et al., 2022)                 |
|                           | VEGF-B gene therapy         | Mitochondrial respiration/capillary| Tumor-bearing mice                | (Monti et al., 2013)                    |
|                           | Statin                      | Endothelial permeability           | –                                 | (Räsänen et al., 2016)                  |
|                           | Irisin                      | EndMT                              | Male C57BL/6J mice                | (Wilkinson et al., 2018)                |
|                           | Pyrroloquinoline quinine    | Autophagy dependent                | HUVECs                            | (Pan et al., 2021)                      |
|                           | Dox                         | Paracrine                          | –                                 | (Jiang et al., 2019)                    |
|                           | Ginsenoside Rg3             | oxidative stress                   | Male SD rats                      | (Lemmens et al., 2006)                  |
|                           |                             |                                    |                                   | (Wang et al., 2015)                     |

(Continued on next page)
| Cell type     | Drug/Agent | Related mechanism                  | model            | Related pathway        | Ref                      |
|--------------|------------|------------------------------------|------------------|------------------------|--------------------------|
| Immune cell  | Ipilimumab | Immune homeostasis disruption      | C57BL/6J Pd1<sup>+/−</sup> mice | TNFα                   | (Michel et al., 2022)    |
|              | Ipilimumab | ICI-related myocarditis           | Cynomolgus Monkeys | Th1, CXCR4             | (Ji et al., 2019)        |
|              | Ipilimumab/| inflammatory cell infiltration    | Pathological specimen of human heart | CD68/CD3               | (Champion and Stone, 2020) |
|              | nivolumab  |                                     |                  |                        |                          |
|              | Ipilimumab | Inflammation                       | Male C57BL/6 mice | MyD88, NLRP3/IL-1β    | (Quagliariello et al., 2020) |
|              | Nivolumab  | irAE                               | BALB/c mice      | CD4<sup>+</sup>, CD8<sup>+</sup> | (Tay et al., 2020)       |
|              | Ipilimumab | cross talk between CM and macrophage | Male C57/BL6 mice | miR-34a-5p            | (Xia et al., 2020)       |
|              | Ipilimumab | CBT                                | Female BALB/c mice | TGFβ                  | (Martin et al., 2020)    |
|              | Latifolin  | Macrophage Polarization            | Male C57BL/6 mice | M1/M2                 | (Zhang et al., 2020)     |
|              | Dox        | Exosome/Pyroptosis                 | C57BL/6J mice    | MyD88, p-P38, and p-JNK | (Ferrante and Leibovich, 2012) |
|              | Dox        | Inflammation                       | Male SD rats     | Estrogen, β-MHC       | (Phungphong et al., 2020) |
|              |            |                                     |                  |                        |                          |
| Platelet     | Clofazimine| platelet activation                | –                | ADP, thrombin          | (Anderson et al., 2018)  |
|              | Sunitinib/ | coronary microvascular dysfunction | CS7BL/6 mice     | PDGFRβ                 | (Chintalagattu et al., 2013) |
AUTHOR CONTRIBUTIONS

WQL and BKZ had the idea for the article, SFX performed the literature search and data collection and drafted the article, WQL, ZHL, YYY, XYL, and JL drafted critically revised the work. All authors modified and approved the final article.

DECLARATION OF INTERESTS

The authors declare that they have no competing interests.

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