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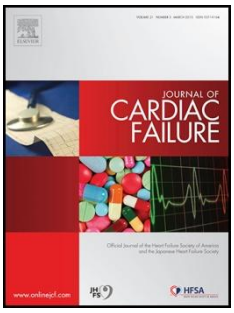

Title: Models of Heart Failure Based on the Cardiotoxicity of Anticancer Drugs

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Models of heart failure based on the cardiotoxicity of anticancer drugs

Short title: Anticancer drugs and models of heart failure

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HIGHLIGHTS

- Heart failure (HF) is a common complication of oncological treatments
- Models of HF can be classified based on the mechanisms of cardiotoxicity of antineoplastic drugs
- Myocyte-intrinsic forms include ROS generation, mitochondrial, DNA and metabolic damages
- Paracrine forms include blockade of ErbB2, VEGFR and PDGFR
- Anthracyclines and trastuzumab can also induce HF by affecting the CPC population

ABSTRACT

Heart failure (HF) is a complication of oncological treatments that may have dramatic clinical impact. It may acutely worsen a patient's condition, or it may present with delayed onset, even years after treatment, when cancer has been cured or is in stable remission. Several studies have addressed the mechanisms of cancer therapy-related HF (CTHF) and some have led to the definition of disease models that hold valid for other and more common types of HF. Here, we review these models of HF based on the cardiotoxicity of antineoplastic drugs, and classify them in cardiomyocyte-intrinsic, paracrine, or potentially secondary to effects on cardiac progenitor cells (CPC). The first group includes HF due to the combination of oxidative stress, mitochondrial dysfunction and activation of the DNA damage response, which is typically caused by anthracyclines, and HF resulting from deranged myocardial energetics, such as that triggered by anthracyclines and sunitinib. Blockade of the neuregulin-1/ErbB4/ErbB2, vascular endothelial growth factor (VEGF)/VEGF receptor and platelet-derived growth factor (PDGF)/PDGF receptor pathways by trastuzumab, sorafenib and sunitinib is proposed as paradigm of CTHF associated with alterations of myocardial paracrine pathways. Finally, anthracyclines and trastuzumab are also presented as examples of antitumor agents that induce HF by affecting the CPC population.

Keywords: heart failure; antineoplastic drugs-induced cardiotoxicity; anthracyclines; receptor tyrosine kinase

INTRODUCTION

Recent advances in antineoplastic treatments have rendered cancer curable in a sizable percentage of subjects, which is likely to further increase in the next years. In many other cases prolonged remission is achieved, leaving patients free of disease for a considerable amount of time. Unfortunately, these improvements take their toll in terms of emerging chronic side effects of antineoplastic agents, which can predominate once a tumor is eliminated or durably controlled [1]. Asymptomatic reduction in left ventricular (LV) function and heart failure (HF) are prototypical complications of cancer therapies that may have long-lasting impact [2]. Much effort has been expended in trying to pinpoint the mechanisms of cancer therapy-related HF (CTHF). This substantial body of work has allowed the identification of potential approaches to tackle the cardiotoxicity of antitumor agents, and additional ones are expected to be investigated in the next future. On the other hand, such an approach has also contributed to uncover important insights into the pathogenesis of HF in general. Among the many mechanisms that have been reported for CTHF, here we focus on those which have proven to be also relevant for other, more common forms of HF. We classify them as cardiomyocyte-intrinsic and paracrine, depending on whether they primarily affect cardiomyocytes or paracrine signals regulating cardiomyocytes, respectively. Furthermore, we propose disturbance of the cardiac progenitor cell (CPC) compartment as a third, stand-alone potential event in CTHF that may apply to other types of HF (Table 1). Each model is discussed referring to the anticancer agents that typically cause it, although it may also explain the cardiotoxicity of other drugs.

CARDIOMYOCYTE-INTRINSIC MECHANISMS

Oxidative stress and DNA damage

Anthracyclines currently constitute key components of chemotherapeutic regimens for the treatment of different adult and pediatric cancers, such as leukemia and lymphomas as well as many solid tumors, including breast cancer [3]. These agents are amongst the most cardiotoxic anti-cancer drugs. Anthracycline cardiotoxicity can manifest acutely, early after infusion, thus requiring either modification or withdrawal of anti-cancer regimens [4]. Luckily, this is a relatively rare complication of chemotherapy, occurring in less than 9% of all patients suffering treated with anthracyclines, and recent evidence clearly demonstrates that anthracycline side effects are usually dose-dependent and more frequently detected within the first year after completing the treatment [5,6]. Pre-existent heart diseases and advanced age represent major risk factors for CTHF and recent studies also highlight the existence of a gender-related predisposition. Interestingly, a significantly higher risk for subclinical cardiotoxicity has been found in females than in males [7-9] and this could be ascribed, at least in cellular models, to testosterone-mediated protection from anthracycline-induced senescence of cardiomyocytes [10] (see Table 2 for a comprehensive list of risk factors).

Cardiotoxicity is a well-known adverse effect of anthracyclines and the research performed in the last 50 years has started to shed light on the molecular pathways involved. From a pathophysiological point-of-view, anthracyclines induce cardiomyocyte death, primarily apoptosis and necrosis, through several molecular mechanisms, including but not limited to induction of oxidative stress, activation of DNA damage responses and impairment of mitochondrial biogenesis and metabolism (Figure 1) [11]. The maladaptive changes occurring in surviving myocytes, as well as those occurring in the extracellular matrix, eventually lead to pathological LV remodeling, with dilatation and impairment of contractility, up to decline in systolic function and development of clinical HF.

Anthracycline-mediated myocyte damage has traditionally been attributed to the production of reactive oxygen species (ROS) with a subsequent increase in oxidative stress, which in turn causes membrane lipid peroxidation, vacuolization, irreversible damage, and myocyte replacement by fibrous tissue [2,3,12-18]. This primarily stems from the susceptibility of anthracyclines to be rapidly reduced to unstable metabolites (such as doxorubicin-semiquinone) which in turn react with oxygen to generate hydrogen peroxide and superoxide. Alternatively, the redox properties of anthracyclines have been ascribed to their ability to chelate free intracellular iron and form iron-doxorubicin complexes which, in turn, react with oxygen and trigger ROS production. Finally, anthracyclines can directly interfere with the activity of major iron-transporting and -binding proteins [19]. On these grounds, doxorubicin has been recently shown to impair the expression of the mitochondrial iron exporter, ABCB8, thus promoting mitochondrial iron accumulation and ROS production [20]. Accordingly, overexpression of ABCB8 *in vitro*, in isolated cardiomyocytes, and *in vivo*, in the hearts of transgenic mice, decreases mitochondrial iron and cellular ROS and protects against doxorubicin-induced cardiomyopathy. Intriguingly, hearts from patients with doxorubicin-related heart dysfunction have significantly higher mitochondrial iron levels than hearts from patients with other types of cardiomyopathies or normal cardiac function [20]. These results thus suggest that mitochondrial iron accumulation and oxidative stress constitute a major hallmark of doxorubicin-induced cardiomyopathy.

A recent study indicates that anthracycline-induced ROS formation may additionally result from the interaction of the drug with the beta isozyme of topoisomerase II (Top2), an enzyme responsible for managing DNA tangles and supercoils, in cardiomyocytes [21] (Figure 1). Of the two Top2 isoforms, Top2-alpha and Top2-beta, Top2-alpha is overexpressed in proliferating cancerous cells, but not in quiescent tissues, and it is thought to be the molecular target of doxorubicin antitumor activity. By binding both Top2-alpha and DNA, doxorubicin forms a ternary Top2-doxorubicin-DNA cleavage complex, which in turn triggers the death of tumor cells. Unfortunately, doxorubicin also interacts with cardiac Top2-beta, the only isoform expressed by

adult mammalian cardiomyocytes, and the Top2-beta-doxorubicin-DNA complex induces DNA double strand breaks, ultimately promoting cardiomyocyte death [14]. The hypothesis that doxorubicin-mediated cardiomyopathy is mediated by cardiomyocyte Top2-beta is supported by studies showing that cardiomyocyte-specific *Top2-beta* deletion protects cardiomyocytes from doxorubicin-induced DNA double strand breaks and eventually results in almost complete protection against the development of doxorubicin-induced progressive heart failure in mice [14].

Mechanistically, it is likely that Top2-beta binding by doxorubicin, and the ensuing DNA break, lead to the activation of the DNA damage response and in turn, of the tumor suppressor protein p53. This enzyme, which is responsible for the activation of DNA repair proteins, can also repress genes involved in mitochondrial biogenesis, such as *Ppargc1*, and oxidative phosphorylation, ultimately leading to defective organelle biogenesis and metabolic failure [14]. Intriguingly, doxorubicin-activated p53 further contributes to this metabolic derangement by inhibiting the proper recycling of dysfunctional mitochondria via autophagy, a catabolic mechanism facilitating degradation of cytoplasmic proteins as well as old/damaged organelles. As a consequence, doxorubicin-injured mitochondria accumulate in the myocardium, leading to increased production of harmful reactive oxygen species (ROS) and ultimately cardiomyocyte death. In support of this view, p53-deficient mice show less decline of cardiac functional reserve after treatment with doxorubicin [22]. Notably, p53-null mice display preserved mitochondrial integrity and cardiac function also with increasing age. These data thus suggest that p53-mediated inhibition of autophagy may represent a major mechanism underlying not only anthracycline-related cardiomyopathies, but heart failure progression in general [22].

Besides p53, anthracyclines can also trigger the mitogen activated protein kinase (MAPK) cascade through reactive oxygen species and Ca^{2+} [23,24]. Notably, within MAPK family, ERK is known to protect cardiomyocytes from apoptosis, whereas p38 MAPK is involved in the induction of cardiomyocyte death [23]. Further studies are awaited to clarify the role of these enzymes and of other uncharacterized signaling pathways in anthracycline-mediated cardiomyocyte damage.

Nevertheless, these data indicate that the molecular mechanisms underlying anthracycline-related cardiomyopathy may be also relevant for other and more common forms of HF and suggest that timely innovative pharmacological interference with the primary mechanisms of cardiac injury, such as p53 activation, may represent a common strategy to fight heart disease.

Deranged myocardial energetics

Among other mechanisms involved in anthracycline-induced cardiotoxicity, recent studies have identified the role of deranged myocardial energetics, expressed by a reduced phosphocreatine/ATP ratio, which precedes LV dysfunction [25]. Indeed, anthracyclines are able to oxidize sulphydryl groups of creatine kinase (CK), diminishing its function, thus lowering myocardial energetics [25] and cauing cardiac dysfunction. More studies on such an interesting mechanism could provide a target for novel preventive therapies. Indeed, overexpression of myofibrillar CK in mice with HF caused by aortic banding significantly improved cardiac function [26], suggesting a key role for CK in HF prevention and treatment. Accordingly, the same group showed that CK overexpression also improved myocardial energetics, contractile function and survival in a mouse model of doxorubicin-induced cardiotoxicity [27]. These results provide novel strategies for limitation of anthracycline-related cardiotoxicity.

Anthracyclines can also alter cardiac energy metabolism by reducing the level of 5' AMP-activated protein kinase (AMPK, involved in the response to energy stress) and phosphorylation of anti-acetyl-CoA carboxylase (ACC), resulting in impairment of fatty acid oxidation [28]. The mechanisms underlying inhibition of AMPK require further investigation.

Interestingly, sunitinib, a tyrosine kinase inhibitor (TKI), has been shown to inhibit AMPK as an off target effect which could lead to ATP depletion and affect contractility [29,30]. In pressure-overloaded mice treated with sunitinib, Chu and coworkers [31] showed opening of the mitochondrial permeability transition pore and marked mitochondrial swelling with derangements of the normal mitochondrial structure in cardiomyocytes. Also, direct sunitinib administration on

different myocardial preparations produces a dose-dependent negative inotropic effect, paralleled by a decline in intracellular Ca2+ and enhancement of ROS generation [32,33]. Interestingly, our preliminary data show that CK might play a role in the modulation of sunitinib myocardial effects [34].

PARACRINE MECHANISMS

Disruption of the NRG-1/ErbB4/ErbB2 system

Cardiotoxicity of anticancer agents targeting ErbB2 is one of the best examples of how cardiac side effects of an oncological treatment have prompted major advances in cardiac biology and pathology, with specific reference to HF.

ErbB2 (also known as HER2/Neu) is a membrane receptor belonging to the epidermal growth factor receptor family, which also includes ErbB1, ErbB3, and ErbB4. It is normally activated by the interaction (dimerization) with another ErbB receptor, which occurs after the latter one is stimulated by a ligand. [35]. In up to 30% of breast cancers, ErbB2 is overexpressed and transmits tumor growth-promoting intracellular signals regardless of the presence of an ErbB ligand (Figure 2) [36]. This has led to the design of ErbB2-targeting drugs, the first-in-class being trastuzumab, a humanized monoclonal antibody against ErbB2 [37]. Unexpectedly, trastuzumab was associated with LV dysfunction and HF in a substantial proportion of treated women in the pivotal clinical trials, with the highest incidence of cardiac complications in patients who concurrently received anthracyclines [38]. This outcome fueled a considerable body of research on the role of ErbB2 in the heart: in a decade, it was discovered that ErbB2 is part of a signaling system fundamental to maintain post-natal cardiac homeostasis.

It is now known that microvascular endothelial cells of the adult myocardium release neuregulin-1 (NRG-1, particularly the NRG-1β isoform) in response to stimuli such as mechanical strain [39,40]. NRG-1 acts on contiguous cardiomyocytes in a paracrine way and triggers the dimerization of ErbB2 with ErbB4, which is followed by a number of cellular events contributing

to cardiac homeostasis and adaptation to stress [41-43] (Fig 2). After the observation that trastuzumab was cardiotoxic in many recipients, mice with cardiac-specific disruption of the NRG-1/ErbB4/ErbB2 system were created via conditional deletion of the *ErbB2* gene [44,45]. These animals proved to be viable and displayed normal cardiac morphogenesis at birth, but developed a dilated cardiomyopathy with systolic dysfunction in early adulthood [44,45]. Therefore, it was postulated that trastuzumab causes cardiomyocyte damage and, eventually HF, by inhibiting the NRG-1/ErbB4/ErbB2 axis in the heart, and that this is more likely to happen if cardiomyocytes are simultaneously exposed to another source of stress, like anthracyclines [46-48]. In principle, the same may be true when other types of cardiac stress are present, e.g. hypertension.

Several criticisms have subsequently been raised to this paradigm, which probably does not fully explain the cardiac side effects of trastuzumab and other ErbB2-blocking agents [49]. Nevertheless, it may still hold valid and – most important - has paved the way for further studies of NRG-1/ErbB4/ErbB2 that have moved the focus from cancer therapy-related HF to HF of any etiology, and new therapeutic perspectives have been opened. For instance, in a mouse model of LV pressure overload, ErbB4 and ErbB2 mRNA and protein were shown to decrease significantly during the transition from compensatory LV hypertrophy to HF [50]. Consistently, ErbB2 and ErbB4 expression and phosphorylation (a marker of receptor activation) was reported to be reduced in LV myocardium from HF patients, compared to that from organ donors [51]. Interestingly, it was also found that LV unloading after LV assist device implantation restored ErbB4 and ErbB2 levels [51,52]. In apparent contrast with these investigations, enhanced phosphorylation of ErbB4 and ErbB2 was observed in pacing-induced HF in dogs [53]. However, the intracellular mediators downstream of ErbB4 and ErbB2, Akt and ERK1/2, were inactivated, pointing anyway to disabled NRG-1/ErbB4/ErbB2 signaling. NRG-1 expression was higher in HF than in control conditions in most studies [51,53].

Overall, these findings suggest that impaired NRG-1/ErbB4/ErbB2 activity is implicated in the pathogenesis of HF at least in two ways: i) HF may ensue from the use of medications blocking

the NRG-1/ErbB4/ErbB2 pathway, like trastuzumab; ii) ErbB4/ErbB2 downregulation and/or uncoupling from intracellular signaling cascades occurs during the natural history of any type of HF in spite of normal or increased NRG-1, possibly contributing to disease progression. Moreover, it has been recently shown that catecholamines, that typically rise with the onset of cardiac dysfunction and following anthracycline treatment [54,55], can enhance ErbB2 expression in cardiomyocytes, thus rendering these cells more "targetable" by trastuzumab, thus vulnerable to the drug toxic effects [56]. These data may support the use of beta-blockers to prevent trastuzumab cardiotoxicity [57]. Indeed, a retrospective study by Seicean et al.indicated that coincidental use of β-blockers was able to lower the incidence of symptomatic HF in breast cancer patients treated with trastuzumab, anthracyclines, or both [58]. Pharmacological prevention with β-blockers is currently being tested in clinical trials [57; 58; 59] with bisoprolol (MANTICORE 101-Breast) [60], carvedilol (NCT01009918), and metoprolol (NCT01434134, NCT00806390) to prevent or treat trastuzumab-induced cardiomyopathy [57]. Interestingly, preliminary data from the PRADA (Prevention of cardiac dysfunction during adjuvant breast cancer therapy) trial presented at the 2015 American Heart Association Scientific Sessions showed that blocking just β1 with metoprolol may not provide sufficient protection [61], thus supporting the use of an unbiased β1- and β2-blocker [56].

Based on the evidence that NRG-1 exerts cardioprotective actions via ErbB4/ErbB2 and that the activity of these receptors is defective in HF, it has been postulated that NRG-1 and NRG-1 analogs may be employed for HF treatment. Intravenous administration of recombinant human NRG-1 and of the glial growth factor 2 (GGF2) isoform of NRG-1β improved cardiac performance and reduced LV dimensions in various animal models of HF [62-64]. Since NRG-1 therapy was beneficial in experimental ischemic HF even when it was started after myocardial infarction, it is conceivable that it induces reverse remodeling of the failing heart and does not merely oppose LV dilatation [62,64]. In addition, NRG-1 appears to directly inhibit cardiac fibroblasts and prevent fibrosis [64].

Early-phase clinical studies showed that infusion of recombinant human NRG-1 is safe, well tolerated, and favorably affects LV volumes and EF until up to 3 months after treatment [65,66]. Nonetheless, it should be noted that systemically administered NRG-1 might serve as growth factor for cancer cells or microfoci. Additional ongoing clinical trials will hopefully address this safety concern and will generate new evidence about the efficacy of NRG-1 in HF [40].

VEGF/VEGF-R and PDGF/PDGF-R blockade

Sunitinib and sorafenib are TKIs that target more than 30 kinases, and this explains their high rate of toxicities. Sunitinib targets VEGF receptors (VEGFR) 1–3, platelet-derived growth factor receptor (PDGFR), c-Kit, FMS-like tyrosine kinase-3, colony-stimulating factor-1 receptor (CSF-1R), and the product of the human RET gene (RET, mutated in medullary thyroid carcinomas/ multiple endocrine neoplasia). It is indicated in metastatic renal cancer and in imatinib-resistant gastrointestinal stromal tumor (GIST) and pancreatic neuroendocrine tumors that cannot be treated with surgery [2,29,32,67]. The main kinases inhibited by clinically relevant concentrations of sorafenib are VEGFR, PDGFR, Raf – 1/B-Raf, c-Kit, and FLT3 in *in vitro* kinase assays. Sorafenib is used to treat patients with advanced renal cell carcinoma after failure of prior therapy with interferon alfa or interleukin-2, or patients that are considered unsuitable for such therapy; it is also indicated for the treatment of liver cancer [2,29,32,67].

Beside the aforementioned AMPK, the fundamental target of these drugs is vascular endothelial growth factor (VEGF) signaling. Since VEGF is both an important regulator of cardiomyocyte function and growth, and of the integrity and expansion of the coronary and systemic circulation (Fig 2) [13,17,29,67-72], not surprisingly inhibiting VEGF signaling can induce cardiotoxic effects, mainly hypertension, thrombo-embolism, LV dysfunction, and HF [73,74]. Indeed, the heart depends on adequate perfusion for its normal function, and, similarly to cancer, relies on the integrity of HIF-1 (a transcriptional activator that is sensitive to cellular hypoxia and mediates many cellular and systemic homeostatic responses to hypoxia [75]) and

VEGF pathways [13,17,29,67-72]. In particular, inhibiting HIF-1 with p53 produced LV dysfunction after chronic pressure overload [24], while conditional expression of a VEGF scavenger led to microvessel rarefaction and myocardial hibernation that could be fully reversed even months after turning off the expression of the scavenger [76,77]. These findings point out that the heart is sensitive to anti-angiogenic drugs, particularly with pressure overload.

Inhibition of VEGF would also be responsible for the most common cardiovascular side effect of antiangiogenic drugs, namely hypertension. Indeed, VEGF signaling is important for the regulation of endothelial function and nitric oxide generation; its inhibition therefore abolishes normal vasodilation [78]. Other effects of VEGF inhibition may include induction of endothelial cell death and rarefaction of resistance vessels [2; 79]. Hypertension is due to mechanisms similar to those involved in the anticancer effects of antiangiogenic drugs, and therefore may also be considered as a marker for these drugs'efficacy [80].

Sunitinib has a high incidence of cardiotoxic effects. Besides its programmed action on VEGF, these important side effects can be also explained by the drug's inhibition of off-target kinases, such as ribosomal S6 kinase (RSK), which brings to subsequent activation of the intrinsic apoptotic pathway [29].

Inhibition of PDGFR could be another mechanism related to sunitinib and sorafenib induced toxicity, since PDGFR also plays a critical role in cell survival and cardioprotection during pathologic stress (Fig 2). Exposure of mice lacking PDGFR (PDGFR-β KO) to afterload stress results in LV dilation, decreased cardiac function, and pulmonary congestion compared with controls. This is also accompanied by increased apoptosis and decreased expression of pro-angiogenic genes [81]. PDGFR inhibition is also known to impair angiogenesis, and leads to microvascular dysfunction through loss of pericytes [82].

Sunitinib and sorafenib share many mechanisms of cardiotoxicity, although sorafenib may have additional effects through inhibition of the Rapidly Accelerated Fibrosarcoma (RAF) kinases that normally promote cell survival [83]. It has been demonstrated in animal models that cardiac-

specific deletion of RAF-1 results in left ventricular (LV) dilatation and reduced contractile function [84].

Interestingly, a recent study addressed additional mechanisms of sorafenib-induced cardiotoxicity. The authors found that mice treated with sorafenib showed a decreased 2-week survival after myocardial infarction (MI) compared to vehicle-treated controls. In particular, sorafenib cardiotoxicity resulted from myocyte necrosis rather than from any direct effect on myocyte function, with surviving myocytes undergoing pathological hypertrophy. Inhibition of c-kit+ stem cell proliferation was also advocated as a potential, exacerbating factor that decreased endogenous cardiac repair [85].

All things considered, it is clear that cardiotoxicity from antiangionenic drugs is a complex side effect achieved by a combination of inhibitions of the activities of different Tyrosine Kinases that have important roles both in tumor cell proliferation and cardiovascular homeostasis (Table 3).

EFFECTS OF CARDIAC PROGENITOR CELLS

Emerging evidence seems to indicate that the cardiotoxicity of anti-cancer drugs is not solely restricted to cardiomyocytes but may also affect resident cardiac progenitor cells [86,87]. Although the heart has long been considered a definitively differentiated organ, without regenerative capacity, some studies have reported the presence of stem cells in the myocardium, known as cardiac progenitor cells (CPCs; see also [88] for extensive review of CPC function in health and disease). These cells seem to be self-renewing, clonogenic, and multipotent, and have been suggested to be implicated in the constant and physiological renewal of myocytes, endothelial cells (ECs), smooth muscle cells (SMCs), and fibroblasts. Furthermore, CPCs could potentially contribute to myocardial regeneration in pathological states. For instance, they regenerate cardiomyocytes and coronary vessels partly restoring the structure of the infarcted myocardium [86]. Nevertheless, although their identification has triggered a first wave of enthusiasm, the actual existence of CPCs and their regenerative capacity have been questioned and are still controversial [89]. In particular, there is

considerable controversy regarding whether c-kit+ cells are even cardiomyocyte progenitors [90,91,92]. Further studies are thus required to conclusively prove the therapeutic potential of these regenerative approaches, also considering other stem cells populations, mainly Sca-1+ cardiac progenitor cells, but also committed mesenchymal precardiomyocytes positive for Nkx2.5 or Isl1 [93].

Despite these controversial views, recent studies have proposed CPCs as an additional cellular target responsible of doxorubicin-induced cardiomyopathy [86] (Figure 3). Doxorubicin has been reported to interfere with the endothelial differentiation capacity of CPCs mediated by the CCL2/CCR2 pathway, as this drug reduces CCL2 expression and promotes the depletion of cardiac erythropoietin (EPO), which binds to CPCs and seems to be responsible for maintaining an active CCL2/CCR2 system [93]. Accordingly, mice exposed to doxorubicin display attenuated CCL2/CCR2 activation as well as reduced EPO levels in the cardiac microenvironment. Of note, mice prone to developing heart failure as a result of reduced cardiac STAT3 expression (cardiomyocyte-restricted deficiency of STAT3) show similar defects, accompanied by increased activity of MMP-12 which is responsible for the proteolytic cleavage of CCL2 [93]. These data thus support the view that deregulation of EPO/CCL2/CCR2 signaling might represent a general mechanism underlying CPC dysfunction in heart failure. In agreement, EPO expression is downregulated by 4-fold in hearts from patients with non-ischemic HF [94]. More importantly, this work envisages the possibility of exploiting EPO supplementation as a therapeutic strategy to reduce the risk of HF not only during anticancer therapies, but also in other disease conditions. EPO treatment in a low dose that does not increase the hematocrit (hematocrit-inactive) is indeed sufficient to upregulate CCL2, restore endothelial differentiation of CPCs, and preserve the cardiac microvasculature and cardiac function in both doxorubicin-treated and STAT3-KO mice [93].

Impaired CPC differentiation may be also responsible of the late-onset doxorubicin-induced cardiotoxicity which represents a major concern in pediatric oncology. Juvenile mice exposed to doxorubicin, using a cumulative dose that does not induce acute cardiotoxicity, display impaired

vascular development, resulting in abnormal vascular architecture in the hearts with less branching and decreased capillary density. This in turn results in an adult heart that is more susceptible to stress as both physiological (exercise) and pathological (myocardial infarction) stress induce late-onset cardiomyopathy in the adult doxorubicin-treated mice. Notably, when subjected to myocardial infarction, doxorubicin-treated mice display fewer progenitor cells in the infarct border zone and fail to increase capillary density in the injured area, thus supporting a model where doxorubicin treatment reduces proliferation and differentiation of the progenitor cells into cells of cardiac lineages [95].

Mechanistically in CPCs, anthracycline exposure has been shown to increase ROS production and induce DNA damage, expression of p53, telomere attrition and apoptosis (Figure 3). In addition, doxorubicin promotes cell cycle arrest at the G2/M transition, by decreasing cyclin D1, cdk4 and phosphorylated Rb levels, in a dose- and time-dependent manner [96]. In agreement, $p16^{INK4a,}$ also known as cyclin-dependent kinase inhibitor 2A, is upregulated in patients who died of doxorubicin-induced heart failure and indicates that the majority of CPCs are senescent [86,96]. Doxorubicin also induces profound changes in global gene expression of CPCs, including genes that are implicated in drug efflux and cell protection from toxic agents, such as the ATP-binding cassette ABC transporter Abcg2/Mdr1, and genes involved in self-renewal and progenitor cell expansion [86,96]. Inhibition of CPC division in combination with accumulation of oxidative DNA damage, growth arrest, cellular senescence, and apoptosis ultimately leads to an almost complete depletion of the CPC pool within the myocardium over a period of 6 weeks after doxorubicin exposure. Intriguingly, supplementation of syngeneic progenitor cells has been proven effective in opposing the progression of doxorubicin cardiotoxicity in a rat model and suggests that CPC repopulation of the depleted myocardium after aggressive chemotherapy may represent a valuable option to rescue the cardiomyopathic heart [96].

Finally, the other cardiotoxic antineoplastic agent, trastuzumab, has also been reported to impair the cardiomyogenic and angiogenic capacities of CPCs. Although clinically relevant

concentrations of trastuzumab have only minor effects on proliferation, apoptosis, or size of the c-kit-positive CPC subpopulation, *in vitro* assays indicate diminished potential for cardiogenic differentiation and impaired ability to form microvascular networks in trastuzumab-treated cells. Consistently, trastuzumab treatment impairs the functional benefits of CPCs injected into the border zone of acutely infarcted mouse hearts [87].

Overall, these findings propose cardiac progenitor cells as one of the cellular targets responsible for chemotherapy-induced cardiomyopathy and envisage a CPC repopulation strategy for therapeutic intervention. Further studies, however, are awaited to clarify the debatable nature and therapeutic potential of this cardiac cell subpopulation.

CONCLUSIONS

Many of anti-cancer drugs induced toxicities share paradigmatic mechanisms of LV dysfunction. New, key cardiovascular signaling pathways involved in myocardial homeostasis have been more deeply understood through the study of the side effects caused by these drugs. Indeed, understanding the relevance of such pathways might prove important in order to study and develop novel therapeutic approaches for heart failure patients and improve longevity and quality of life in cancer patients. It is likely that along with the growing development of targeted cancer therapies, novel toxicities will be discovered [67], but in the same time these unfortunate findings may unveil unexplored myocardial signalings, thus allowing for parallel development in novel potential HF treatment strategies, including, for instance, genetic manipulation, miRNAs, gene transfer [13,16,27,97-106].

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DISCLOSURES

All authors have materially participated in the article preparation, All authors have approved the final article.

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FIGURE LEGENDS

Figure 1: Cardiomyocyte-intrinsic molecular mechanisms underlying anthracycline cardiotoxicity.

Anthracyclines, such as doxorubicin (DOX), lead to cardiotoxicity by promoting the production of reactive oxygen species (ROS), via direct (unstable DOX metabolites, such as doxorubicin-semiquinone, react with oxygen and generate hydrogen peroxide and superoxide) and indirect (DOX chelates free iron and modulates the activity/expression of major iron-transporting/binding proteins) mechanisms. Alternatively, DOX can interact with cardiomyocyte topoisomerase 2β (Top2β), an enzyme responsible for managing DNA tangles and supercoils, thus inducing DNA double-strand breaks. DNA damage, in turns, activates the tumor suppressor protein p53, which is responsible for the activation of DNA repair proteins, but also of repression of genes involved in mitochondrial biogenesis/recycling and oxidative phosphorylation pathways. Finally, DOX can accumulate within mitochondria of cardiomyocytes and exacerbates metabolic failure of these organelles. Altogether, these molecular events contribute to cardiomyocyte death and ultimately to cardiac dysfunction.

Abbreviations: ROS, reactive oxygen species; TOP2β, topoisomerase 2β.

Figure 2: Paracrine molecular mechanism underlying main TKI cardiotoxicity.

Adapted from [13], [17] and [106].

Therapies targeting paracrine signaling network at several levels with antibodies such as trastuzumab or with TKIs are able to perturb the potentially protective action of VEGF, PDGF and NRG in the myocardium. In particular, neuregulin-1 (NRG1) binding brings to dimerization between the neuregulin receptor ErbB4 and its co-receptor ErbB2. ErbB2 and ErbB4 phosphorylate each other on tyrosyl residues activating several signaling pathways, including those involving Akt that inhibits apoptotic signaling and promotes cellular hypertrophy, while ERK is able to phosphorylate transcription factors thus promoting gene transcription.

Figure 3. Effects of anthracyclines on cardiac progenitor cell function.

Anthracyclines, such as doxorubicin (DOX), interfere with different functions of CPCs. DOX impairs the endothelial differentiation capacity of CPCs by downregulating CCL2/CCR2 signaling, either directly (DOX reduces CCL2 expression) or indirectly (DOX promotes the depletion of a key regulator of CCL2/CCR2, cardiac erythropoietin). Furthermore, DOX dampens CPC proliferation by downregulating key controllers of cell cycle progression, such as cyclin D1, cdk4 and phosphorylated Rb. Finally, DOX-dependent ROS production and DNA damage contribute to the activation of the tumor suppressor protein p53 ultimately driving to telomere attrition and apoptosis. Abbreviations: ROS, reactive oxygen species; CPCs, cardiac progenitor cells; CCL2, chemokine ligand 2; CCR2, C-C chemokine receptor type 2; Rb, retinoblastoma protein; cdk4, cyclin-dependent kinase 4.

Table 1. Chemotherapeutic agents commonly associated with cardiotoxicity

Proposed mechanisms	**Chemotherapeutic drug**
Cardiomyocyte intrinsic	Anthracyclines, sunitinib
Paracrine	Trastuzumab, sunitinib, sorafenib
Effects on cardiac progenitor cells	Anthracyclines, trastuzumab

Table 2. Risk factors for cancer-related heart failure

Risk factor	**References**
Age	[6]
Cumulative anthracycline dose	[6], [7], [8]
End-chemotherapy LVEF	[6]
Family history of coronary artery disease	[6]
Younger age at treatment	[7]
Sex female	[6], [7], [9]

Abbreviations: LVEF, left ventricular ejection fraction.

Table 3 (modified from [67])

Cardiovascular role of the main kinases inhibited by antiangiogenic drugs

Kinases	**Role in Cardiovascular system**	**Inhibitors**
VEGFR	Contribution to cardiomyocyte function and growth and to the integrity and expansion of the coronary and systemic circulation; stimulation of endothelial growth, migration, and survival	Sunitinib and sorafenib
PDGFR	Contribution to cell survival and cardioprotection during stress conditions, regulation of angiogenesis	Sunitinib and sorafenib
AMPK	Energy production	Sunitinib
RAF	Promoting cell survival	Sorafenib
c-kit	Contribution to homing of CSC to sites of post-MI injury, CSC differentiation, and cardiomyocyte terminal differentiation.	Sunitinib and sorafenib
ribosomal S6 kinase (RSK)	signals survival through inhibitory phosphorylation of the pro-apoptotic factor BAD	Sunitinib