The tell-tale heart: molecular and cellular responses to childhood anthracycline exposure

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Lindsey ML, Lange RA, Parsons H, Andrews T, Aune GJ. The tell-tale heart: molecular and cellular responses to childhood anthracycline exposure. Am J Physiol Heart Circ Physiol 307: H1379–H1389, 2014. First published September 12, 2014; doi:10.1152/ajpheart.00099.2014.—Since the modern era of cancer chemotherapy that began in the mid-1940s, survival rates for children afflicted with cancer have steadily improved from 10% to current rates that approach 80% (60). Unfortunately, many long-term survivors of pediatric cancer develop chemotherapy-related health effects; 25% are afflicted with a severe or life-threatening medical condition, with cardiovascular disease being a primary risk (96). Childhood cancer survivors have markedly elevated incidences of stroke, congestive heart failure (CHF), coronary artery disease, and valvular disease (96). Their cardiac mortality is 8.2 times higher than expected (93). Anthracyclines are a key component of most curative chemotherapeutic regimens used in pediatric cancer, and approximately half of all childhood cancer patients are exposed to them (78). Numerous epidemiologic and observational studies have linked childhood anthracycline exposure to an increased risk of developing cardiomyopathy and CHF; often decades after treatment. The acute toxic effects of anthracyclines on cardiomyocytes are well described; however, myocardial tissue is comprised of additional resident cell types, and events occurring in the cardiomyocyte do not fully explain the pathological processes leading to late cardiomyopathy and CHF. This review will summarize the current literature regarding the cellular and molecular responses to anthracyclines, with an important emphasis on nonmyocyte cardiac cell types as well as those that mediate the myocardial injury response.

anthracyclines; congestive heart failure; cardiomyopathy; cardiomyocytes; fibroblast; extracellular matrix

APPROXIMATELY 325,000 SURVIVORS of pediatric cancer currently reside in the United States, and 1 in 340 young adults is a cancer survivor (60). Anthracyclines are components of most curative combination chemotherapy regimens for childhood leukemia, lymphoma, and solid tumors, and approximately half of all newly diagnosed pediatric cancer patients will be treated with an anthracycline-containing regimen (78). Many of these individuals have, or are at risk of developing, chronic illnesses related to their childhood cancer treatments that adversely affect their long-term survival (6, 34, 55, 72, 93, 96). Cardiovascular disease and secondary malignancies are the two leading causes of premature death in pediatric cancer survivors (6).

Reports of cardiovascular outcomes in pediatric cancer survivors treated with anthracyclines have focused primarily on clinical outcomes, current preventive strategies, or recommendations for clinical management (9, 22, 25, 38, 43, 44, 48, 68, 77–83, 125, 126, 140). The rationale for our review is based on the following three observations. First, the clinical outcomes in anthracycline-exposed individuals are not completely explained by the known molecular effects of anthracyclines on the cardiac myocyte. Second, to our knowledge no recent review has focused on anthracycline cardiotoxicity in the nonmyocyte cell populations of the heart and those that mediate the myocardial injury response. Third, additional laboratory models are urgently needed to better understand the pathogenesis of heart disease in this population, and these models must account for both the observed clinical outcomes and the events that occur over time in the collective myocardial cell types that are responsible for pathological events at the organ level. Therefore, our review will 1) briefly summarize the clinical cardiovascular outcomes to provide the necessary clinical context and 2) focus on the molecular events that occur in...
the cell types that comprise myocardium and those that
direct the responses that occur with injury. Because cardiac
myocytes have historically been the central focus of anthra-
cycline cardiotoxicity research, this review will be heavily
weighted toward molecular events in them. Nevertheless,
our overall goal is to highlight all myocardial cell types and
injury response mechanisms that collectively guide the path-
ological development of disease in anthracycline-exposed
patients. These cell types include immune cells that are part
of the inflammatory reaction and resident cardiac fibroblasts
that are part of the wound healing response and produce
extracellular matrix (ECM). Developing a more balanced
approach to understanding myocardial anthracycline-in-
duced injury may facilitate the identification of novel targets
to ameliorate the risk for cardiac disease in pediatric cancer
survivors.

Cardiovascular Outcomes in Long-Term Survivors of
Pediatric Cancer Exposed to Anthracyclines

Cardiovascular complications in anthracycline-exposed pe-
diatric cancer survivors may manifest clinically as congestive
heart failure (CHF), ischemic heart disease, stroke, or cardiac
mortality. Cardiac imaging in these subjects may demonstrate
functional or anatomic abnormalities such as cardiomyopathy,
diastolic dysfunction, and valvular disease. These collective
outcomes are important to consider in the context of under-
standing the interrelated events in anthracycline-exposed myo-
cardial cell populations and the development of relevant pre-
clinical laboratory models of anthracycline cardiotoxicity. A
number of studies have demonstrated an increased risk for
these cardiovascular outcomes, most of which have used the
National Cancer Institute-funded U24 resources of the Child-
hood Cancer Survivor Study (CCSS). The CCSS is a multi-
institutional effort that has collected treatment and outcome
data on 14,357 long-term childhood cancer survivors and 3,899
sibling controls (72). Oeffinger and colleagues (96) used data
from this cohort to show that the relative risk (RR) of cardio-
vascular disease is markedly increased in long-term pediatric
survivors compared with matched sibling controls, including
CHF (RR = 15.1), stroke (RR = 9.3), and coronary artery disease
(RR = 10.4). Importantly, Mulrooney and colleagues (93) completed a study using the CCSS cohort in
which the standardized mortality ratio (SMR) for cardiac death
was 8.2 times higher than expected. These findings have been
reinforced by new analysis of existing human cancer patient
clinical registries by our team (manuscript in preparation.).
These analyses provide further confirmation that childhood
cancer survivors are at significantly higher risk of cardiovas-
cular mortality compared with the general population (Fig. 1).
Examining cardiovascular outcomes in a population-based co-
hort of childhood cancer survivors enrolled in the Surveil-
 lance, Epidemiology, and End Results Program (SEER) from 1980 to
1989, we found significantly higher cardiac mortality over time
in survivors compared with the general population (Fig. 1A).
Although the SMR for cancer survivors appears to decrease
over time, it remains at least 10 times higher in long-term
survivors compared with the general population. The lower
SMR over time is likely the result of aging of both
survivors and the general population over time, when the
incidence of cardiac events increases in both groups (Fig. 1B).

Cardiac death in children and young adults is extremely rare,
and any event drives large increases in the SMR compared
with the general population of the same age. The important point
remains that even 20 to 30 years after diagnosis, survivors of
childhood and young adult cancers remain more than three
times more likely to experience cardiac mortality compared
with the general population.

Studies using a variety of imaging modalities show that
cardiac anatomic and functional characteristics are frequently
abnormal in long-term survivors of pediatric cancer who have
no clinical symptoms of cardiac disease. Lipshultz and col-
leagues (81) assessed echocardiographic outcomes in survivors
of childhood cancer and noted lower left ventricular mass, wall
thickness, contractility, and fractional shortening in anthracy-
cline-exposed individuals compared with those who did not
receive an anthracycline. In 277 adult survivors in the CCSS
survey, Brouwer and colleagues (19) showed significantly
higher rates of systolic and diastolic dysfunction in survivors
compared with sibling controls as assessed by echocardiogra-
phy.
Other studies have used more sensitive imaging methods to screen for subclinical cardiovascular complications in the survivor population. For example, Armstrong and colleagues (7) recently used cardiac MRI to study 108 anthracycline-exposed pediatric cancer survivors and found that 14% had a left ventricular ejection fraction of \( <50\% \) that was classified as normal by cardiac ultrasonography. Subclinical cardiac dysfunction [i.e., abnormal regional wall motion (138) and myocardial performance index (108)] has also been identified by echocardiographic studies of anthracycline-treated children, especially in those who received higher cumulative doses of the drug.

The association between anthracycline exposure in childhood and the development of cardiac dysfunction later in life is well documented. Because of these studies, current recommendations are not to exceed anthracycline doses of 450 mg/m\(^2\). However, the underlying molecular mechanisms that explain the late onset of symptoms have not been fully elucidated.

### Etiology of Cardiomyopathy in Anthracycline-Exposed Patients

Anthracycline exposure in childhood initiates a pathological progression that may culminate in dilated cardiomyopathy in adulthood (64). In contrast to almost all other forms of cardiomyopathy, the major myocardial changes that occur after early anthracycline exposure are predominantly found in the interstitial areas and do not result in extensive hypertrophy. Importantly, fibrosis is a major histologic change that occurs in survivors who have received anthracycline chemotherapies (10). Bernaba and colleagues (10) reviewed medical records from 10 patients who had a history of anthracycline exposure and available cardiac tissue samples (9 had cardiac transplantation as a result of anthracycline-induced heart failure). Histologic analysis revealed significant interstitial fibrosis in all 10 patients without hypertrophy.

The molecular and cellular mechanisms that direct the development of anthracycline-induced cardiomyopathy remain largely unknown. With the combination of knowledge of pathological progression with the range of cellular and molecular effects, it is possible to speculate on novel targets and therapies for intervention. A number of studies have used existing knowledge to test protective strategies. A summary of these strategies is listed in Table 1. To identify new therapeutic strategies, it will be imperative to continue research aimed at elucidating the underlying molecular events and testing novel inhibitors in the context of the unique pathology of anthracycline-induced cardiac injury. This review will summarize what is currently known about the cell-specific effects and molecular events induced by anthracyclines in the cardiovascular system and heart.

### Table 1. Drugs and strategies used to prevent anthracycline-induced cardiotoxicity

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Therapy</th>
<th>Target or Mechanism</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Deplete iron needed for ROS production</td>
<td>Dexrazoxane*</td>
<td>Iron chelator</td>
<td>(49, 50, 76, 84, 85, 131)</td>
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<tr>
<td></td>
<td>Deferiprone#</td>
<td>Iron chelator</td>
<td>(3)</td>
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<tr>
<td></td>
<td>Monohydroxyethylrutoside#</td>
<td>Iron chelator</td>
<td>(20, 21)</td>
</tr>
<tr>
<td></td>
<td>Pyroxyl 2-chlorobenzoyl hydrazine#</td>
<td>Iron chelator</td>
<td>(120)</td>
</tr>
<tr>
<td>Countertack ROS effects</td>
<td>Amifostine#</td>
<td>Antioxidant</td>
<td>(13, 14)</td>
</tr>
<tr>
<td></td>
<td>CoQ10#</td>
<td>Antioxidant</td>
<td>(29)</td>
</tr>
<tr>
<td></td>
<td>Didox#</td>
<td>Antioxidant</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>Garlic extract#</td>
<td>Antioxidant</td>
<td>(2)</td>
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<tr>
<td></td>
<td>Glutathione-S-transferase#</td>
<td>Antioxidant</td>
<td>(71)</td>
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<tr>
<td></td>
<td>HO-3867#</td>
<td>Antioxidant</td>
<td>(30)</td>
</tr>
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<td></td>
<td>N-acetylcysteine#</td>
<td>Antioxidant</td>
<td>(94)</td>
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<td></td>
<td>Probucol#</td>
<td>Antioxidant</td>
<td>(36, 74, 75, 115)</td>
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<td></td>
<td>Resveratrol#</td>
<td>Antioxidant</td>
<td>(35)</td>
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<td></td>
<td>Schisandrin B#</td>
<td>Antioxidant</td>
<td>(24, 137)</td>
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<tr>
<td></td>
<td>Tempol#</td>
<td>Antioxidant</td>
<td>(90)</td>
</tr>
<tr>
<td></td>
<td>Telmisartan - angiotensin II blocker#</td>
<td>Antioxidant</td>
<td>(47)</td>
</tr>
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<td></td>
<td>Trimetazidine#</td>
<td>Antioxidant</td>
<td>(124)</td>
</tr>
<tr>
<td></td>
<td>Vitamin E*</td>
<td>Antioxidant</td>
<td>(47, 122)</td>
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<tr>
<td>Prevent apoptosis or regulate mitochondria</td>
<td>Adiponectin#</td>
<td>Antiapoptosis</td>
<td>(88)</td>
</tr>
<tr>
<td></td>
<td>Bosentan#</td>
<td>Antiapoptosis</td>
<td>(12)</td>
</tr>
<tr>
<td></td>
<td>Endothelin-converting enzyme#</td>
<td>Mitochondrial regulation</td>
<td>(89)</td>
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<td></td>
<td>PARP inhibitors#</td>
<td>Antiapoptosis</td>
<td>(98)</td>
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<tr>
<td></td>
<td>Phosphodiesterase-5 inhibitor#</td>
<td>Antiapoptosis</td>
<td>(63)</td>
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<td>Pifithrin-α#</td>
<td>Antiapoptosis - anti-p53</td>
<td>(87)</td>
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<td></td>
<td>Thrombopoietin#</td>
<td>Antiapoptosis</td>
<td>(23)</td>
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<tr>
<td>Regulate inflammatory response</td>
<td>Erythropoietin#</td>
<td>Antioxidant and anti-inflammatory</td>
<td>(4)</td>
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<td></td>
<td>FGF-2#</td>
<td>Cytokine</td>
<td>(116)</td>
</tr>
<tr>
<td></td>
<td>Ilprost#</td>
<td>Synthetic prostaglandin</td>
<td>(95)</td>
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<td>Miscellaneous or unknown</td>
<td>ACE inhibitors*</td>
<td>↓Blood pressure/modify ECM remodeling</td>
<td>(15, 16, 51, 109)</td>
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<tr>
<td></td>
<td>Administration rate</td>
<td>↓Peak serum dose</td>
<td>(11, 57, 82)</td>
</tr>
<tr>
<td></td>
<td>Berberine#</td>
<td>Unknown</td>
<td>(144)</td>
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<tr>
<td></td>
<td>Liposomal delivery*</td>
<td>Selective delivery to tumors to limit off-target exposure</td>
<td>(104)</td>
</tr>
<tr>
<td></td>
<td>Exercise*</td>
<td>↑Antioxidant production and reduce ECM remodeling</td>
<td>(35, 56, 62)</td>
</tr>
<tr>
<td></td>
<td>Metalloporphyrin peroxynitrite decomposition catalyst#</td>
<td>Anti-nitric oxide</td>
<td>(97)</td>
</tr>
<tr>
<td></td>
<td>Statins*</td>
<td>Antioxidant and anti-inflammatory</td>
<td>(113)</td>
</tr>
</tbody>
</table>

ROS, reactive oxygen species; CoQ10, coenzyme Q10; PARP, poly(ADP-ribose) polymerase; ACE, angiotensin-converting enzyme. *Clinical evidence. #Preclinical evidence.
**Cell-Specific Myocardial Response to Anthracyclines**

The major mechanisms of anthracycline-induced myocardial damage that have been explored focus on the iron-dependent generation of reactive oxygen species (ROS) and the induction of cardiomyocyte apoptosis (25), with subsequent loss of myocytes and replacement fibrosis (31). Whereas these clearly important mechanisms explain the early loss of cardiac myocytes, they do not fully explain the complex series of events that occurs later when chemotherapy is completed or the events that occur in other cardiac cells—such as fibroblasts and invading immune cells—that are central mediators of the myocardial injury response (39). Additionally, anthracyclines may be arrhythmogenic during acute infusion (5, 46, 66, 103), injure vascular endothelial cells lining the coronary arteries and endocardium (28, 54, 117, 148), and affect cardiac progenitor cells (CPCs) (17, 31).

The wide-ranging effects of anthracyclines on individual myocardial cell types are summarized in Fig. 2. As depicted in Fig. 2, much more information exists regarding the effects of anthracyclines on the myocyte than on all of the other cell types combined. Emerging data from clinical studies and experiments using cell types and animal models with specific defects in the injury response strongly suggest an important role for these distinct cell types in anthracycline-induced cardiac injury. This review will summarize what is currently known about the molecular responses to anthracyclines in myocytes, endothelial cells, CPCs, and the cells coordinating the cardiac injury response.

**Myocytes.** An extensive amount of experimental data regarding the mechanism of action of anthracyclines in myocytes has been collected, with most obtained in cultured mouse or rat cardiomyocyte cell lines isolated from animals ranging from neonatal age into adulthood. Whereas the generation of ROS has long been a known biological response to anthracyclines, a diverse array of other cellular pathways has also been implicated. However, the interactions among these pathways, their effects on other cell types in the myocardium, and the ECM remain largely unexplored.

**GENERATION OF REACTIVE OXYGEN SPECIES.** In cultured cardiomyocytes, antioxidants protect against oxidative stress induced by doxorubicin (32). The Wallace laboratory (146) used an in vivo model of anthracycline exposure in adult male Sprague-Dawley rats to show elevated ROS formation in isolated cardiomyocytes that persisted for 5 wk after exposure.
was discontinued. Subsequent studies showed that disruption of redox pathways alters the deleterious response to anthracyclines. Turakhia and colleagues (127) used both small interfering RNA knockdown of heat shock protein 27 (HSP27) in cultured cardiomyocytes and heat shock transcription factor (HSF)-1/−/− cells to show that HSP27 protects against doxorubicin damage by preserving catalytic recycling required for maintaining appropriate redox balance. In cofactor for oxidase NADPH oxidase (Nox2)-deficient mice that lack NADPH oxidase, an important ROS source in myocytes, reduced ROS generation results in abrogated cardiac dysfunction, decreased myocyte apoptosis, and interstitial fibrosis (145). Metabolic studies using 13C isotope labeling in cultured cardiomyocytes demonstrated that oxidative metabolism was significantly increased during anthracyclines exposure, implying a relative increase in ROS (121). Finally, the Ren laboratory (134) used ventricular myocytes to show that doxorubicin-induced contractile dysfunction is mediated via p38 MAPK-dependent oxidative stress mechanisms (134).

APOTOPSIS, DNA DAMAGE, AND DNA REPAIR PATHWAYS. The cytotoxic mechanisms of anthracyclines have been extensively studied in vitro and in vivo tumor model systems. These studies demonstrate that anthracyclines damage DNA via intercalation and inhibition of topoisomerase II, an enzyme that relieves torsional strain on the DNA backbone during replication. This results in DNA strand breaks, induction of the DNA damage and repair pathways, and in the event the damage cannot be repaired initiation of the apoptotic response. Importantly, functional topoisomerase II has recently been implicated in the cardiotoxic response to doxorubicin. Zhang and colleagues (142) developed a mouse model with a cardiomyocyte-specific inducible knockout of the topoisomerase IIβ gene to show that doxorubicin-induced cardiotoxicity is abrogated in the absence of functional topoisomerase. In contrast, a recent article published by the Pommier laboratory (65) showed that absence of mitochondrial topoisomerase I resulted in impaired mitochondrial function and enhanced toxicity to doxorubicin. In vitro and in vivo studies have also shown that numerous cellular intermediates involved in the apoptotic response are altered in cardiomyocytes during anthracycline treatment. These include cytochrome c, Bcl-2-to-Bax ratio, and phosphorylation of p300/CREB binding protein (27, 101). In addition, caveolin 1 and 3 are required for the apoptotic response to doxorubicin (132). Alternatively, induction of cell survival pathways such as the WNT-1 pathway has been shown to block cardiomyocyte death in response to doxorubicin (130). Furthermore, the DNA repair and damage responses appear to be central to the mechanism of action of anthracycline-induced cardiotoxicity and have important functional significance. This is supported by all of the following: 1) acute doxorubicin cardiotoxicity is associated with p53-inhibited induction of mammalian target of rapamycin (147); 2) HSP27 (an important mediator of the ROS pathway) regulates p53 transcriptional activity in cultured cardiomyocytes and upregulates p21 to initiate cell cycle arrest (129); and 3) anthracycline-induced cardiotoxicity is abrogated in whole body p53-null mice. Interestingly, cardiomyocyte-specific ablation of p53 in mice using conditional knockdown techniques is not sufficient to block cardiac dysfunction or reduce cardiac fibrosis (37). Finally, alterations in pathways that respond directly to DNA damage have been studied in the context of anthracycline-induced myocyte toxicity. These studies indicate the following: 1) deficiency in poly(ADP-ribose) polymerase, an enzyme that repairs DNA single strand breaks, results in protection against doxorubicin-induced damage (123) and 2) deficiency in breast cancer susceptibility gene-2 protein, a tumor suppressor protein, results in exaggerated cardiomyocyte apoptosis and cardiac failure in mouse models of anthracycline exposure (114). These observations highlight the overall importance of cellular DNA repair mechanisms in the response to anthracycline exposure. However, mechanisms have not been elucidated that explain why deficiency of some DNA repair pathways result in enhanced sensitivity to anthracyclines, whereas others are protective. Thus a greater understanding of how these pathways affect the response to anthracyclines in individual cell types in the myocardium may provide important further insights.

NEUREGULIN/ERBB SIGNALING AXIS. Neuregulin-1 is an important growth and survival factor that exerts its function via the Erb-phosphatidylinositol 3-kinase-AKT pathway in the cardiomyocyte. Numerous in vitro studies have explored its role in the response to anthracyclines. These studies show the following: 1) treatment with neuregulin-1 protects myocytes from anthracycline-induced apoptosis (40); 2) HSP90 stabilizes ErbB2 protein following anthracycline exposure to protect against myocyte apoptosis (41); 3) heterozygous knockout of the neuregulin-1 gene in mice exacerbates heart failure caused by doxorubicin exposure (86); and 4) blocking the neuregulin axis with antibodies to ErbB2 (e.g., trastuzamab) initiates a similar apoptotic response to that obtained with daunorubicin exposure (107). Additionally microRNA-146a overexpression is induced by doxorubicin exposure and mediates cell death via interactions with the Erb-neuregulin signaling axis (53).

ALTERATIONS IN TRANSCRIPTION FACTORS. A variety of studies have investigated the roles that individual transcription factors play in the cardiomyocyte response to anthracyclines. Krishnamurthy and colleagues (69) showed that ablation of transcription factor HSF-1 results in decreased expression of the multidrug resistance transporter P-glycoprotein and protection of cardiomyocytes from doxorubicin injury. Anthracyclines downregulate GATA4, an important survival factor (26, 99, 111), and decrease nuclear factor of activated T-cells 5 mRNA (58).

DISRUPTION OF MYOFILAMENTS AND CALCIUM TRAFFICKING. A number of changes to myocyte filaments occur in response to anthracyclines exposure. In cultured neonatal rat myocytes, dose-dependent ultrastructural changes induced by anthracyclines include disorganization and depolymerization of actin filaments (73). Deng and colleagues (33) investigated cardiotoxicity in dystrophin-deficient mice and found them to be more sensitive to doxorubicin-induced cardiotoxicity than matched control mice. More recently, the Maier laboratory (110) showed that doxorubicin exposure stimulates Ca/calmodulin protein kinase II activation with increased calcium leak from the sarcoplasmic reticulum.

ACTIVATION OF THE UBIQUITIN-PROTEASOME PATHWAY. The ubiquitin-proteasome system is responsible for degradation of many cellular proteins and plays an essential role in homeostasis (105). In animal models, ubiquitin-proteasome activity is increased in cardiomyocytes exposed to anthracyclines (70).
Other studies demonstrate stimulation of 20S proteasomes with doxorubicin exposure and exacerbation of doxorubicin-induced cardiac dysfunction in mice when the E3-ubiquitin ligase Nrdp1 is overexpressed (143).

**Endothelial cells.** Several recent clinical studies have implicated vascular endothelium as an important target of the cardiovascular changes induced by anthracycline exposure. In two separate pilot studies, Mulrooney and colleagues (91, 92) studied 25 long-term survivors of Hodgkin’s lymphoma and 24 survivors of childhood osteosarcoma. Blood samples from these groups of patients exhibited evidence of vascular inflammation, dyslipidemia, and early atherogenesis. In a similar study, Brouwer and colleagues (18) studied 277 adult survivors of pediatric cancer who had received cardiotoxic therapies including anthracyclines. They observed an increase in arterial wall thickness, suggesting changes induced by exposure to systemic chemotherapy. In addition, Jenei and colleagues (61) studied 96 long-term survivors of pediatric cancer and noted that these patients had increased vascular stiffness. Zsary and colleagues (148) measured endothelin-1 levels (a critical regulator of cardiac performance) in 20 one-year survivors of lymphoma. They observed a decrease in plasma endothelin-1 levels that correlated with decreased ejection fraction and cardiac function. Collectively, these clinical studies suggest systemic chemotherapies such as anthracyclines can have deleterious effects on vascular endothelial cells and possibly contribute to long-term cardiac effects in survivors.

The above clinical observations are further supported by substantial evidence from numerous preclinical studies that have investigated the cellular and molecular responses induced by anthracycline exposure in human endothelial cells. In contrast to clinical studies, Bayed-Ahmed and colleagues (112) observed an increase in plasma endothelin-1 in rats treated with anthracyclines. The discrepancy between the clinical and preclinical observations may be attributed to the time at which endothelin-1 was measured; in the clinical study by Zsary, endothelin-1 was quantified one year after completion of anthracycline treatment, whereas in the preclinical study measurements were taken at the immediate end of the treatment. While the direction of the change remains to be determined, the fact that endothelin-1 is changing in both clinical and preclinical models suggests that it might be an important regulator of anthracycline-induced cardiomyopathy.

Hoch and colleagues (52) observed impaired endothelial differentiation in cells isolated from mice treated with doxorubicin. In addition, levels of erythropoietin in the cardiac microenvironment of these mice were reduced and microvascular and endothelial differentiation were restored with supplementation of an erythropoietin derivative. Yamac and colleagues (139) treated rats with anthracyclines and observed extensive degenerative changes in aorta endothelium in nuclei, ribosomes, and basement membrane.

Using bovine aortic endothelial cells, Wang and colleagues (133) observed a time- and dose-dependent activation of nuclear factor-κB (NF-κB). Activation of NF-κB and cell apoptosis were significantly decreased by adding glutathione peroxidase to reduce ROS. The findings suggest that in endothelial cells, anthracyclines induce NF-κB leading to apoptosis, in contrast to cancer cells where activation of NF-κB is antiprototic. Finally, Wu and colleagues (135) examined the effects of anthracycline exposure on the small coronary vessels of rats.

In the endothelial cells that line these vessels, they noted an increase in proapoptotic caspase-3, Bax, and Fas, as well as a decrease in antiapoptotic Bcl-2. Collectively, these observations suggest that anthracyclines induce apoptosis in vascular endothelial cells, possibly leading to increased risk of cardiovascular disease later in life.

**Cardiac progenitor cells.** A number of recent studies suggest that events in CPCs are an important contributing factor in the development of anthracycline-induced cardiomyopathy. De Angelis and colleagues (31) demonstrated that anthracycline treatment depletes the cardiac stem cell pool and that cardiac dysfunction can be restored by injecting cardiac stem cells systemically. In a separate study by the same group, CPCs grown in culture and exposed to doxorubicin were observed initiating the DNA damage response and undergoing apoptosis (100). At later time intervals, shortened telomeres and senescence were observed. In addition, the progeny of doxorubicin-treated CPCs exhibited premature expression of p16 (INK4a), an important marker for cellular senescence. This senescence phenotype is further supported by evidence published by Spallarosa and colleagues who exposed cord blood endothelial progenitor cells to low doses of doxorubicin and observed several important changes including increased SA-b-gal activity, decreased telomeric repeat binding factor 2, chromosomal abnormalities, enlarged cell shape, and disarrangement of F-actin stress fibers (117).

A recent review summarizes new observations linking cardiac toxicity from tyrosine kinase inhibitors (an emerging new class of anticancer agents) to cellular targets in CPCs and possibly other myocardial cell types (102). Collectively, these studies point to CPCs as an important cell type to study in the context of anthracycline-induced cardiomyopathy.

**The injury response:** invading immune cells, fibroblasts, and ECM. The injury response in the heart is mediated by resident cardiac fibroblasts and is known to include complex interac-

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**Table 2. Future research priorities**

<table>
<thead>
<tr>
<th>Current Deficiency</th>
<th>Attributes of Preclinical Laboratory Models Needed</th>
<th>Clinical Trial Development</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lack of pediatric focus</td>
<td>Acute exposure in young animals with long latent period before evaluation</td>
<td>Organized prospective trials need to test long-term efficacy of novel cardioprotective interventions</td>
</tr>
<tr>
<td>Current models do not account for latent period between exposure and adverse cardiac outcomes</td>
<td>Animal models that incorporate exposure in young animals and evaluation of cellular and functional parameters as they age</td>
<td>Validate biomarkers</td>
</tr>
<tr>
<td>Myocardial cell responses poorly understood, particularly for nonmyocyte cell types</td>
<td>Acute exposure models that differentiate responses by cell types and can be used to identify serum biomarkers</td>
<td>Test interventions that slow pathological progression to overt cardiac disease</td>
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tions between invading immune cells and the ECM (39). The known myocardial effects of doxorubicin with respect to these events are summarized below.

**INVADING IMMUNE CELLS.** Endomyocardial biopsy specimens obtained from patients exposed to anthracyclines exhibit myocarditis-like changes with prominent invasion by lymphocytes (42). Zhang and colleagues (141) described the presence of dendritic cells in the hearts of rats treated with doxorubicin. More recently, the innate immune system has been implicated in the mechanism of anthracycline-induced cardiotoxicity with Toll-like receptor-4 deficiency in mice abrogating doxorubicin cardiotoxicity (106).

**THROMBOSPONDIN.** The matricellular protein thrombospondin 1 is known to preserve ECM. Using a thrombospondin-1 knockout mouse model, van Almen and colleagues (128) showed that absence of thrombospondin increases doxorubicin-induced cardiomyocyte damage, disruption of ECM, and mortality in treated mice.

**MATRIX METALLOPROTEINASES.** Matrix metalloproteinases (MMPs) are proteins that enzymatically alter the ECM following cardiac injury and collectively play a major role in the cardiac remodeling process. Recent studies emphasize the importance of ECM remodeling following doxorubicin exposure. Their findings include the following: 1) circulating MMPs can be detected in the serum of rats chronically exposed to doxorubicin (59); 2) proteomic analyses indicate cystatin C increases with anthracycline exposure and inhibits cathepsin B, resulting in increased myocardial fibronectin and collagen (119, 136); 3) increased circulating levels of ECM components are present in anthracycline-exposed animals (119, 136); and 4) alterations in tissue and circulating MMPs levels occur following exposure to anthracyclines (8, 45, 67, 118).

**Summary and Future Research Priorities**

Long-term survivors of pediatric cancer suffer from high rates of cardiovascular disease and cardiac mortality due to exposure to cardiotoxic chemotherapy and radiation in childhood. Anthracyclines are one of the most commonly used classes of chemotherapeutic agents in pediatric oncology, and their administration is associated with adverse cardiovascular outcomes that include CHF, cardiomyopathy, vascular disease, and stroke. Importantly, the widely held views regarding the molecular mechanisms that result in myocardial damage are often focused on the cardiomyocyte, although events in this solitary cell type do not fully explain how anthracycline exposure during childhood results in the clinical outcomes observed decades later. Collectively, these observations lay the foundation for developing the future research priorities we propose in Table 2. Consideration of the molecular events in response to anthracyclines in the various cell types that comprise myocardium is critical to identifying biomarkers of cardiotoxicity and novel cardioprotective therapeutics. In addition, a systematic understanding of how anthracyclines affect all myocardial cell types will allow for the development of novel hypotheses that are focused on elucidating the temporal and cellular relationships that occur in the heart following anthracycline exposure. Understanding these relationships will facilitate development of preclinical laboratory models and new strategies for detection, prevention, and management of cardiac disease in the large number of pediatric cancer survivors previously exposed to anthracyclines. The clinical epidemiologic observations made regarding the long-term cardiac outcomes in survivors of pediatric cancer and the array of myocardial cellular events induced by anthracyclines summarized in this review provide the foundation for a concerted multidisciplinary approach.

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**REFERENCES**

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