

This Review is part of a thematic series on **Cardiovascular Toxicities/Effects of Anticancer Therapeutics**, which includes the following articles:

Introduction to Cardiotoxicity Review Series [*Circ Res.* 2010;106:19–20]

Molecular Mechanisms of Cardiovascular Toxicity of Targeted Cancer Therapeutics [*Circ Res.* 2010;106:21–34]

The Vulnerability of the Heart As a Pluricellular Paracrine Organ: Lessons From Unexpected Triggers of Heart Failure in Targeted Erbb2 Anticancer Therapy [*Circ Res.* 2010;106:35–46]

Emerging Anticancer Therapeutic Targets and the Cardiovascular System: Is There Cause for Concern?

Cardiovascular Disease: Hidden Killer in Adult Survivors of Childhood Cancer

*Thomas Force, Guest Editor*

## Emerging Anticancer Therapeutic Targets and the Cardiovascular System Is There Cause for Concern?

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**Abstract:** The race for a cure to cancer continues, fueled by unprecedented discoveries of fundamental biology underlying carcinogenesis and tumorigenesis. The expansion of the target list and tools to approach them is moving the oncology community extraordinarily rapidly to clinical trials, bringing new hope for cancer patients. This effort is also propelling biological discoveries in cardiovascular research, because many of the targets being explored in cancer play fundamental roles in the heart and vasculature. The combined efforts of cardiovascular and cancer biologists, along with clinical investigators in these fields, will be needed to understand how to safely exploit these efforts. Here, we discuss a few of the many research foci in oncology where we believe such collaboration will be particularly important. (*Circ Res.* 2010;106:1022-1034.)

**Key Words:** cancer therapeutic targets ■ cardiovascular system ■ cardiac effect/cardiotoxicity ■ chemotherapy

Cancer cell proliferation, apoptosis, angiogenesis, invasion, and metastasis are regulated by an interconnecting network of cellular signaling pathways involving extracellular ligands, transmembrane receptors, intracellular signaling protein kinases, and transcription factors. The biology of tumors is further modified by factors such as epigenetic regulators of chromatin structure, and pathways that regulate protein stability such as molecular chaperones and ubiquitin–proteasome pathways. Insights into the molecular mechanisms that mediate intracellular processes have exposed many novel cancer targets against which therapeutic agents have been or are in development. The opportunity to improve outcomes for cancer patients is enormous. The experience with trastuzumab and tyrosine kinase inhibitors outlined by others in this review series highlights how very

well-designed targeted therapeutics can have untoward effects on the heart because of poorly understood or previously unknown roles of these same targets in the heart. As molecular cardiology lunges forward, we are now in a position to make speculations and predictions about what possible effects a new targeted therapy might have on the heart and vasculature. In this review we discuss pathways and processes being targeted in cancer, and speculate why these cancer targets should be on the minds of the cardiovascular clinical and research community.

### Therapies Targeting Paracrine Signaling Systems

The regulation of tumor growth, like other tissues, relies on cell–cell paracrine and endocrine signaling systems that

Original received October 14, 2009; revision received February 5, 2010; accepted February 22, 2010.

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*Circulation Research* is available at <http://circres.ahajournals.org>

DOI: 10.1161/CIRCRESAHA.109.211276

coordinate tumor and vascular growth. The success of cancer therapies targeting erbB2 receptor tyrosine kinase and the vascular endothelial growth factor (VEGF) receptor have fueled the development of therapies aimed at other signaling systems. The effects of these erbB2 and VEGF targeted therapies on cardiovascular function highlights the fact that most signaling systems are not tissue specific. These findings also provide important insights into cardiovascular function. For example, the consistent finding that VEGF-targeted therapy is often associated with a rise in blood pressure teaches us an interesting lesson: VEGF is an important modulator of blood pressure. Similarly, the effect of erbB2-targeted therapy on cardiac function points to a role for this signaling system in regulation of myocardial structure and/or function. As new therapies focused on other signaling systems are developed and introduced to clinical trials, we are certain to learn more. Moreover, the experience with trastuzumab should remind us that when these systems are expressed in the heart, and are known to play a role in cardiac development and/or in response to cardiac injury, potential cardiac effects should be considered in trial design. In our review, we have chosen to discuss only a few of the many targets of cancer therapies under development targeting paracrine signaling system.

**The c-Met Receptor Tyrosine Kinase**

c-Met (cDNA MNNG-HOS erbb transforming gene) is a membrane-spanning receptor tyrosine kinase that bears considerable similarity to the epidermal growth factor receptor (EGFR) family and regulates several biological activities in normal and malignant tissue.<sup>1</sup> Hepatocyte growth factor (HGF), the only known c-Met ligand,<sup>2</sup> induces receptor autophosphorylation with subsequent activation of intracellular pathways critical for regulation of cell proliferation, survival, invasion, etc.<sup>3</sup> Dysregulation of HGF/c-Met signaling in tumors can occur via multiple mechanisms including mutations to the proto-oncogene MET that increase c-MET expression, and gene amplification of both ligand and receptor.<sup>4</sup> These observations led to the development of strategies to target HGF and c-Met including kinase inhibitors and antibodies much like the strategies developed to target erbB2.

C-Met-targeted therapies may have adverse effects on the cardiovascular system. Like the erbB2/4 receptor tyrosine kinases and their ligand neuregulin, HGF/c-Met plays a critical role in myocardial development,<sup>5</sup> and regulates cardiovascular angiogenesis.<sup>6</sup> In the adult heart HGF/c-Met interactions also regulate cardiac hypertrophy and remodeling, in part, because of activation of the local renin-angiotensin system.<sup>7</sup> In addition, c-Met interacts with the Akt pathway to regulate myocyte survival in the adult heart following injury.<sup>8,9</sup> Myocardial ischemia and reperfusion induce HGF expression in various organs including the heart.<sup>10</sup> Moreover, HGF/c-Met signaling plays a role in capillary endothelial cell regeneration in the ischemic heart.<sup>9</sup>

Several HGF/c-MET inhibitors are currently in early stages of clinical development. AMG-102 is a fully humanized monoclonal antibody against HGF. Early phase I results have been reported.<sup>11</sup> The finding of dose-limiting toxicity of dyspnea and/or hypoxia as well as fatigue should raise some

Non-standard Abbreviations and Acronyms	
<b>Bcl-2</b>	B-cell CLL/lymphoma 2
<b>c-Met</b>	cDNA MNNG-HOS erbb transforming gene
<b>DNMT</b>	DNA methyltransferase
<b>EGFR</b>	epidermal growth factor receptor
<b>FAK</b>	focal adhesion kinase
<b>HDAC</b>	histone acetyltransferase
<b>HGF</b>	hepatocyte growth factor
<b>Hh</b>	Hedgehog
<b>Hsp</b>	heat shock protein
<b>IAP</b>	inhibitors of apoptosis proteins
<b>IGF</b>	insulin-like growth factor
<b>IGFR</b>	insulin-like growth factor receptor
<b>IP3</b>	inositol 1,4,5-trisphosphate
<b>MDM2</b>	mouse double minute 2
<b>miRNA</b>	microRNA
<b>mTOR</b>	mammalian target of rapamycin
<b>NF-κB</b>	nuclear factor κB
<b>PARP-1</b>	poly(ADP-ribose) polymerase
<b>PI3K</b>	phosphoinositide 3-kinase
<b>PLC</b>	phospholipase C
<b>Raf</b>	Ras-activated factor
<b>Ras</b>	rat sarcoma
<b>Src</b>	sarcoma
<b>STAT</b>	signal transducer and activator of transcription
<b>TNF</b>	tumor necrosis factor
<b>UP-S</b>	ubiquitin-proteasome system
<b>VEGF</b>	vascular endothelial growth factor

concerns more than possible cardiovascular effects of this agent, particularly as duration of exposure increases during phase II trials. Similar concerns should accompany the development of XL-880, an oral small-molecule inhibitor of c-MET kinase, particularly because this inhibitor has activity against other signaling systems including VEGFR2 (vascular endothelial growth factor receptor 2), platelet-derived growth factor receptor (PDGFR), kit (V-kit hardy-zuckerman 4 feline sarcoma viral oncogene homolog), FLT3 (fms-related tyrosine kinase 3), Tie-2 (tyrosine kinase with immunoglobulin and egf factor homology domains 2), and Ron (recepteur d'origine nantais).<sup>12,13</sup> In the early phase trials with XL-880 common side effects included hypertension and fatigue.<sup>12,13</sup> Several other small molecule inhibitors targeting c-Met are in the pipeline. Although our understanding of how the HGF/c-Met pathways regulates cardiac structure, function, and response to injury is incomplete, it would seem prudent to closely monitor patients who are receiving these experimental therapies, particularly those with any history of cardiac disease or significant risk factors.<sup>14</sup>

**Notch and Hedgehog Signaling**

Notch and Hedgehog (Hh) have been recently validated in preclinical studies as novel cancer targets. There are 4 homologs of Notch which play critical roles in the function

and physiology of several tissues.<sup>15</sup> Notch signaling promotes communication between adjacent cells, regulating cell-fate, tissue organization, cell proliferation and apoptosis.<sup>16</sup> Ligand-induced Notch receptor activation requires  $\gamma$ -secretase that releases the intracellular domain from the plasma membrane and allows it to translocate into the nucleus where it activates its target genes.<sup>17</sup> Transgenic mice overexpressing active Notch1, Notch3, or Notch4 homologs develop mammary carcinoma.<sup>18,19</sup> Furthermore, a recent clinical study reported that the expression level of Notch1, Notch3, and JAG-1, one of the Notch ligands, were inversely correlated with the overall clinical outcomes in breast cancer patients.<sup>20</sup> In a breast cancer cell line, Notch appears critical for the development of resistance to trastuzumab.<sup>21</sup> These and other observations led to the development of therapeutic agents targeting Notch for the treatment of cancer. Among the several options to block Notch signaling, small molecule  $\gamma$ -secretase inhibitors (GSIs) appear promising.<sup>22,23</sup>

Notch signaling also regulates cardiovascular development and homeostasis. Mutations in Notch signaling elements cause cardiac abnormalities in mice and humans, demonstrating an essential role for Notch during cardiac morphogenesis. Notch also plays a role in regulating cardiac hypertrophy, cardiomyopathy and heart failure.<sup>24–27</sup> In addition to Notch-1, Notch-4 has also been detected in highly vascularized adult tissues including heart.<sup>28</sup>  $\gamma$ -Secretase inhibitor substrates include  $\beta$ -amyloid precursor protein, E-cadherin, CD44, ErbB-4, ephrin-B1, as well as all 4 Notch receptors and their ligands.<sup>29</sup> Therefore,  $\gamma$ -secretase inhibitors do not selectively disrupt Notch, but will also effect other molecules which also play important roles in the adult heart.

Hh is also involved in embryonic development and adult tissue homeostasis<sup>30</sup> and can be abnormally activated in cancer cells.<sup>31</sup> Smoothed (SMO) is a transmembrane protein that localizes to the cell membrane when hedgehog ligands (Sonic, Indian, or Desert Hh) bind to the cell surface receptor Patched1 (Ptch1). Surface localization of SMO initiates a signaling cascade that leads to activation of glioma-associated (Gli) transcription factors.<sup>32</sup> Hh is also involved in coronary development and coronary neovascularization in the adult heart through induction of VEGF and angiopoietin.<sup>33</sup>

As cancer therapies targeting Notch and hedgehog signaling move forward into clinical trials, the role these signaling systems play in cardiovascular development and in the adult heart suggests that caution is warranted. In addition to regulating cardiac development and function, Notch and hedgehog are involved in large vessel<sup>34</sup> and valvular formation.<sup>35</sup> Thus assessment of large vessel and valvular function, which are not the typical cardiovascular end points of clinical trials, would seem appropriate in long-term clinical studies of Notch/Hedgehog inhibitors.

### Insulin-Like Growth Factor Receptor Pathway

The insulin-like growth factor receptor (IGFR) interacts with multiple circulating ligands including insulin-like growth factor (IGF)-I, IGF-II, and insulin.<sup>14</sup> On formation of a heterotetramer, the receptor complex recruits and activates several downstream signaling pathways.<sup>36,37</sup> The IGF-1 re-

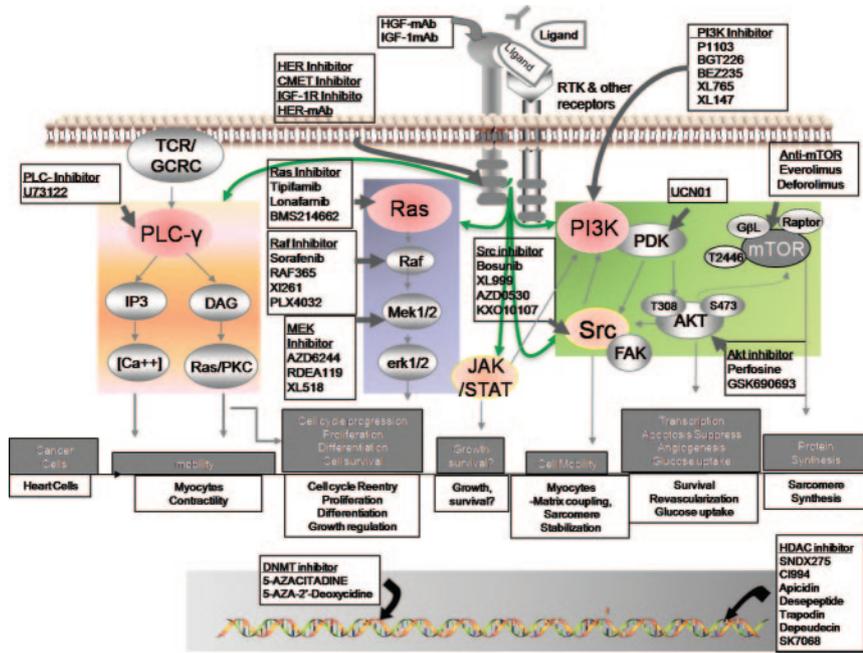
ceptor (IGF-1R) regulates fetal development<sup>38</sup> and growth of many organs including the heart.<sup>39</sup> The IGF-1R has been implicated in the development and maintenance of malignant phenotypes, and disruption of IGF-1R signaling does inhibit cancer cell growth and motility both in vitro and in vivo.<sup>40,41</sup> Increasing levels of circulating IGF are associated with higher risk for colon and breast cancer, and aberrant activation of IGF-1R was also associated with worse prognosis in many neoplasms, including multiple myeloma, prostate cancer, non-small cell lung cancer, and renal cell cancer.<sup>42,43</sup> Aside from the IGF-1R, abnormally activated insulin receptor (IR) by insulin or IGF-II stimulation enhances mitogenesis in cancer cells.<sup>44</sup>

The insulin receptor/IGF-1R are expressed in the heart and play an important role in cardiac development<sup>45</sup> and cardiac hypertrophy.<sup>46</sup> Activation of mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase (PI3K)/AKT/mTOR (mammalian target of rapamycin) through IGF-1R protects the heart from apoptosis during reperfusion injury and improves recovery after heart failure.<sup>47</sup> IGF-1R is also involved in physiological cardiac hypertrophy induced by exercise.<sup>48</sup> Heterozygote cardiac specific knock out of IGF-1R does not alter baseline function but is critical for heart recovery after myocardial infarction.<sup>48</sup> IGF/IGF-1R has been implicated in cardiac regeneration in normal and pathological conditions.<sup>49</sup> There is growing evidence that IGF is involved in cardiac myocyte survival in pathological conditions.<sup>50</sup> IGF-I-deficient states have been associated with development of heart failure as well as ischemic heart disease.<sup>51</sup> Thus the potential for cardiac toxicity should be taken into consideration during the clinical development of IGF targeted therapies.

Most IGF-targeted cancer therapies undergoing clinical development appear to be directed against the IGF-1R, and several of these are in phase II clinical trials. No specific cardiac events have been reported to our knowledge, although fatigue was the most commonly reported side-effect in trials with 2 fully humanized monoclonal antibody antagonist of IGF-1R, followed closely by hyperglycemia.<sup>52,53</sup> Fatigue can obviously be attributed to multiple etiologies, including cardiac dysfunction. Whether a cardiac etiology is the cause of the reduced activity tolerance is not clear at this point. Careful cardiac monitoring seems warranted as these trials proceed.

### Phosphoinositide-Specific Phospholipase C Isozyme

Intracellular signaling networks of molecules with crosstalk and redundancy transmit the signals activated by cytokines, hormones, and growth factors from the extracellular environment to the nucleus. These intracellular signaling pathways can be divided into 4 modules with crosstalk among any 2 of them: phospholipase (PL)C pathway, PI3K pathway, rat sarcoma (Ras)/ras activated factor (Raf)/mitogen-activated protein kinase pathway, and STAT (signal transducer and activator of transcription) pathway (Figure 1). Inhibition of intracellular signaling can suppress cell proliferation, differentiation or angiogenesis induced by diverse extracellular stimuli. In addition there are many other proteins in these pathways which have been targeted in cancer therapy, includ-



**Figure 1. Therapies are being developed for cancer targeting multiple signaling pathways that are known to be present in heart cells.** Many pathways are involved in the proliferation, angiogenesis, and differentiation in neoplasms and are also expressed in the cardiovascular system. Membrane-bound human epidermal growth factor receptors (HER), c-MET, and IGF-1R mediate mitogenic signals from extracellular ligands, such as epidermal growth factor (EGF), HGF, and IGFs, respectively. The Ras/Raf/MEK (mitogen-activated protein/extracellular signal-regulated kinase)/ERK (extracellular signal-regulated kinase) (mitogen-activated protein kinase [MAPK]) and PI3K/Akt/mTOR pathways are major intracellular axes that regulate intracellular signaling traffic. Janus kinase (JAK)/STAT are critical components of many cytokine receptor systems, regulating growth, survival, differentiation and pathogen resistance. PLC/IP3/Ca<sup>2+</sup>, or PLC/diacylglycerol (DAG)/Ras (PKC), non kinase signaling pathways, can regulate cellular motility, differentiation and proliferation. Intracellular signaling kinases are net-

works of those molecules with that transmit the signals activated by cytokines, hormones, and growth factors from the extracellular environment to the nucleus. DNMTs and histone deacetylases (HDAC) are “epigenetic switches” that modulate the expression of oncogenes and tumor suppressor genes. Agents under development that target these signaling proteins are indicated in boxes. Although the investigation of the role of these pathways in the heart lags behind the cancer studies, there are known biological effects that should be considered as clinical trials with targeted agents proceed.

ing mTOR, glycogen synthase kinase-3, phospholipase D, etc, many of which have been specifically discussed in depth by Force and colleagues in this review series.

Rapid hydrolysis of the membrane phospholipid PI(4,5)P2 (phosphatidylinositol 4,5-bisphosphate) is triggered by the interaction of many extracellular signaling molecules such as growth factors or hormones with their cell surface receptors. This reaction is catalyzed by PLC isozymes and results in the generation of 2 intracellular messengers: inositol 1,4,5-trisphosphate (IP3) and diacylglycerol.<sup>54</sup> PLC-γ, one of six isotypes of PLC isozymes, is the most studied of the PLC family. Homozygous disruption of PLC-γ1 gene caused an embryonic lethal phenotype with many defects.<sup>55</sup> PLC-γ1 is ubiquitously expressed. Developmental defects for all organs<sup>55</sup> including the heart were observed in the PLC-γ1<sup>-/-</sup> mice.

In addition to cellular proliferation, PLC-γ1 plays an essential role in cellular differentiation<sup>56</sup> and cell motility.<sup>57</sup> Moreover, PLC-γ1 may be involved in growth factor-induced mitogenesis in normal cells<sup>58</sup> and tumorigenesis in cancer<sup>59</sup> by interacting with proteins via a lipase-independent mechanism. Activation of PLC-γ1 is modulated by both protein tyrosine and serine/threonine kinases.<sup>54</sup> All of these characteristics make PLC an attractive target for cancer treatment. Small molecules targeting PLC developed so far are in preclinical testing. Vinaxanthone, Q12713, hispidospermidine, caloporoside, CRM-51005, CRM-51006 have shown anticancer activity.<sup>60</sup>

Cardiomyocytes closely regulate IP3 responses. IP3 binding to IP3 receptor on the peri-nuclear membrane causes localized Ca<sup>2+</sup> signals that influence transcriptional activity.<sup>61</sup> This precise regulation of IP3 levels implies an important physiological

function for IP3 in cardiomyocytes. The importance of PLC in regulating myocardial calcium handling and contractility will likely become clearer as cancer therapies targeting PLC move forward.

### Epigenetic Modulators As Targets for Cancer Therapy

Epigenetics is the study of regulation of gene expression that is not attributable to any alteration in the DNA base nucleotide sequence.<sup>62</sup> Methylation of DNA sequences, as well as reversible modification of histone proteins by acetylation, methylation, phosphorylation, and mono- and polyubiquitination regulates chromatin structure and accessibility of chromatin to the transcriptional machinery. Epigenetic modifications turn sets of genes “on” and “off”, and thereby regulate the status of a cell.

In cancers, epigenetic modifications are known to regulate tumor-suppressor genes or oncogenes, leading to dysregulated cellular proliferation and apoptosis.<sup>63,64</sup> Epigenetic regulation of cellular phenotype and proliferation is also recognized to play a role in malignant transformation and tumorigenesis,<sup>65,66</sup> with DNA methylation and histone modifications being the most developed targets for anticancer therapy.<sup>67,68</sup> DNA methylation occurs at specific DNA sequences, termed CpG islands, and are most often located near promoters.<sup>69</sup> DNA methyltransferases (DNMTs) regulate promoter activity and effectively silence large groups of genes.<sup>69,70</sup> For example, hypermethylation of CpG island near the promoter of the estrogen gene<sup>71</sup> and methylation of the APC and APC2 gene in colon and rectal cancer tissue has been reported in more than 50% of patients.<sup>72</sup>

DNA strands are wrapped around histones to form nucleosomes. Expression of genes is silenced when the packing of histones condenses chromatin.<sup>73</sup> Acetylation of histone by histone acetyltransferases (HATs) at amino-terminal tails loosens histone binding and promotes expression of local genes.<sup>74,75</sup> Conversely, deacetylation by histone acetyltransferases (HDACs) results in tighter histone-DNA interactions.<sup>76,77</sup> Growing evidence supports a therapeutic potential for inhibition of HDACs against diseases including cancer<sup>78</sup> and cardiac hypertrophy.<sup>79,80</sup> Interestingly, DNA methylation and histone deacetylation appear to synergistically regulate gene silencing through direct interactions between DNMTs and HDACs.<sup>76</sup>

Genes that regulate tumor response to cytotoxic therapy are also regulated by epigenetic mechanisms. Recent studies on promoter methylation and histone acetylation status of transport proteins responsible for multidrug resistant phenotype (MDR1) in cancer cells have revealed hypomethylation of the promoter region of MDR1.<sup>81</sup> Furthermore, high H3 and H4 histone acetylation, and low mRNA expression of DNMTs and HDACs appear to contribute to the upregulation of MDR1 in these cells.<sup>81,82</sup>

It is becoming clear that epigenetic modifications occur in and contribute to the pathogenesis of cardiovascular conditions including cardiac hypertrophy and heart failure.<sup>83</sup> HDAC4 and HDAC5 are predominantly expressed in the heart. HDAC5 or HDAC9 deletion leads to cardiac hypertrophy.<sup>84,85</sup> HDACs have been implicated in both inhibition<sup>83,86</sup> and activation<sup>87,88</sup> of cardiac hypertrophy. Differential chromatin scanning methods applied to myocardium from humans with dilated cardiomyopathy reveals global epigenetic changes involving many canonical signaling pathways implicated in the regulation of myocardial function.<sup>89</sup> Although the functional significance of these changes to the development of heart failure remains to be determined, these observations raise concerns that cancer therapies targeting epigenetic modifiers such as HDACs, histone acetyltransferases, and DNMTs will have consequences for myocardial gene expression patterns, and myocardial structure and function.

DNMTs are inhibited by several drugs that have been in clinical use for some time. 5-azacytidine (azacitidine; Vidaza) and 5-aza-2-deoxycytidine (decitabine; Dacogen), the 2 most studied DNMT inhibitors, were developed initially as cytotoxic agents to treat leukemia.<sup>90</sup> Interestingly, these agents at low doses over a long period of administration are predominantly epigenetic modulators, instead of being cytotoxic.<sup>90</sup> These nucleoside analogs replace cytosine during DNA replication and are, thus, only active during the S phase. The DNA/nucleoside-analog complex then stoichiometrically binds to and inhibits DNMTs. Hydralazine and procainamide, drugs used for the treatment of hypertension and arrhythmias respectively, have recently been recognized to be nonnucleoside DNMT inhibitors.<sup>91</sup> Though cardiotoxicity of the inhibitors to DNMT and HDAC have not been reported, close cardiac monitoring seems warranted during clinical trials.

### Transcription Factor Targets in Cancer

Cellular signaling pathways induce gene expression by activating specific transcription factors for genes involved in

cellular proliferation, survival and differentiation. Two transcription factors currently targeted in cancer that we have chosen to focus on are nuclear factor (NF)- $\kappa$ B and p53, which regulate cell inflammation, angiogenesis, proliferation and apoptosis in cancer cells.

NF- $\kappa$ B regulates the expression of several genes, such as cyclooxygenase 2,<sup>92</sup> inducible nitric oxide synthase (iNOS),<sup>93</sup> tumor necrosis factor (TNF),<sup>94</sup> interleukin-6,<sup>95</sup> cytokines,<sup>96</sup> and BCL-2-related protein (BCL-X).<sup>97</sup> Two NF- $\kappa$ B inhibitors have been developed to the point of phase I clinical trials: RTA 402 and DHMEQ. RTA 402 is a dual inhibitor for NF- $\kappa$ B and inhibitor  $\kappa$ B- $\alpha$  (IKK)/STAT,<sup>98,99</sup> and it induces nuclear erythroid 2 p45-related factor (Nrf2)-mediated transcription of antioxidant proteins which helps suppress tumor proliferation.<sup>100</sup> DHMEQ inhibits the translocation of NF- $\kappa$ B into the nucleus as well as the DNA binding to its components; it is also a potent chemo- and immuno-sensitizing agent, which in combination with cytotoxic therapeutics results in significant reversal of resistance, and induction of cell death in tumors.<sup>101</sup>

P53 is a tumor suppressor and a transcription factor that plays an important role in regulating cell proliferation and apoptosis in cancer. Interestingly, a novel transcription-independent pathway of p53 was recently identified and proapoptotic functions mediated by the cytoplasmic pool of p53 have been revealed.<sup>102</sup> P53 participates directly in the intrinsic apoptosis pathway by interacting with the multidomain members of the Bcl-2 (B-cell CLL/lymphoma 2) family to induce mitochondrial outer membrane permeabilization.<sup>102</sup> Nutline, an inhibitor of the interaction between p53 and MDM2 (mouse double minute 2), binds selectively to the pocket of MDM2, resulting in increased p53 levels. Nutline is an effective inhibitor in cancer cell lines and in mice with various types of tumors when administered orally.

NF- $\kappa$ B and p53 play several roles in the heart that warrant consideration as cancer therapies targeting these proteins develop. Cardiac remodeling is a determinant of the clinical progression of heart failure, and so slowing or reversing remodeling through targeting these prohypertrophic signaling pathways may provide a therapeutic strategy in heart failure. Cardiac-specific inhibition of NF- $\kappa$ B attenuates Angiotensin II induced left ventricular hypertrophy in vivo.<sup>103</sup> NF- $\kappa$ B and inhibitor  $\kappa$ B- $\alpha$  (I $\kappa$ B $\alpha$ ) are phosphorylated in the heart after abdominal aortic constriction (AAC) induced pressure overload. This implicates the NF- $\kappa$ B signaling as an important pathway in the myocardial hypertrophy induced by AAC. Recently a study on exercise has indicated that p53 promotes aerobic metabolism and exercise capacity by inducing mitochondrial genes and signaling pathways in a tissue-specific manner.<sup>104</sup> P53 also activates the mitochondrial death pathway and provokes apoptosis of ventricular myocytes independently of DNA binding and de novo gene activation.<sup>105</sup> So far cardiac side effects have not been observed with NF- $\kappa$ B inhibitors and inhibitors of the interaction between p53 and MDM2, but considering the central role of these proteins in the heart, close cardiac monitoring during clinical trials with therapies targeting this pathway seems warranted.

## MicroRNAs

MicroRNAs (miRNA) are a class of small noncoding endogenous RNA that have generated excitement in the clinical and scientific communities.<sup>106</sup> The discovery that miRNA expression is frequently dysregulated in cancer has uncovered an entirely new repertoire of molecular factors upstream of gene expression, which warrants extensive investigation to further elucidate their precise role.<sup>107</sup> The involvement of miRNAs in the initiation and progression of tumors holds great potential for diagnostic and therapeutic strategies in the management of patients. There is obviously great demand to identify novel miRNAs, the function of their targets, and to understand their role in carcinogenesis their true potential as therapeutic agents.

Several miRNAs have been studied in developing and adult heart. Studies in cell systems and mice indicate the miRNAs may play a pivotal role in altering global signaling networks during progression of cardiac pathology, and dysregulation of miRNAs could promote cardiac dysfunction. Many miRNAs are significantly altered in dilated cardiomyopathy compared to nonfailing controls.<sup>50</sup> miR7 and miR378 are downregulated during initiation of cardiac dysfunction and in end-stage heart failure.<sup>108</sup> Conversely, miR-145 increases significantly in end stage cardiomyopathy.<sup>108</sup> It is intriguing that recent studies in cancer have shown that these same miRNAs play an important role in cancer cells. MiR378 is known to enhance cell survival and promotes tumor growth and angiogenesis.<sup>109</sup> MiR-145 also acts as an antioncomir common to gastrointestinal tumors.<sup>110</sup> We speculate that targeting these miRNAs in cancer might have adverse effects on cardiovascular function.

## Enhancing Cancer Cell Death by Targeting Apoptotic and DNA Repair Pathways

### Targeting Apoptosis

The molecular regulation of cell survival, and the prevention of cell death, has revealed a rich array of targets for cancer therapy. Dysfunction in the regulation of anti- or proapoptotic genes confers enhanced survival and chemotherapy resistance to cancer cells. Cancer therapies are being developed that activate both the intrinsic and extrinsic pathways of cell death.

Several chemotherapeutic agents have been developed to target Bcl-2 and mitochondrial permeability. They consist of molecules that mimic the BH3 (Bcl-2 homology domain 3) and several small molecule inhibitors, such as AT 101, Obatoclax, and ABT-263, are in phase I or II clinical trials.<sup>111</sup> Another strategy is the augmentation of Smac activity<sup>112</sup> by inhibition of IAP (inhibitors of apoptosis protein) family members, including XIAP (x-linked IAP), IAP1/2, and survivin. To date, the most successful target is survivin, an inhibitor of apoptosis which has been implicated in several types of neoplasia.<sup>113</sup> LY2181308 is an antisense molecule targeting survivin currently in phase I testing. YM-155 is a small-molecule inhibitor that targets survivin transcription. YM155 sensitizes NSCLC cells to radiation both in vitro and in vivo, and this effect is likely attributable, at least in part, to

the inhibition of DNA repair and enhancement of apoptosis that result from the downregulation of survivin expression.<sup>114</sup>

Recent studies on survivin in conditional knockout mice in the heart and knockdown/overexpression experiments in myocytes demonstrated that survivin plays a crucial role in controlling cardiomyocyte number during embryonic development and adult life. Survivin promotes cardiomyocyte replication and has been proposed as a strategy to induce myocardial regeneration.<sup>115</sup> Similarly, Bcl-2 overexpression reduces myocyte death in stressed conditions and rescues myocardial dysfunction observed in congenital cardiomyopathy models, such as desmin null mice. These studies raise concerns that targeting survivin and BH3 (Bcl-2 homology domain 3) proteins in cancer may have adverse cardiac effects.

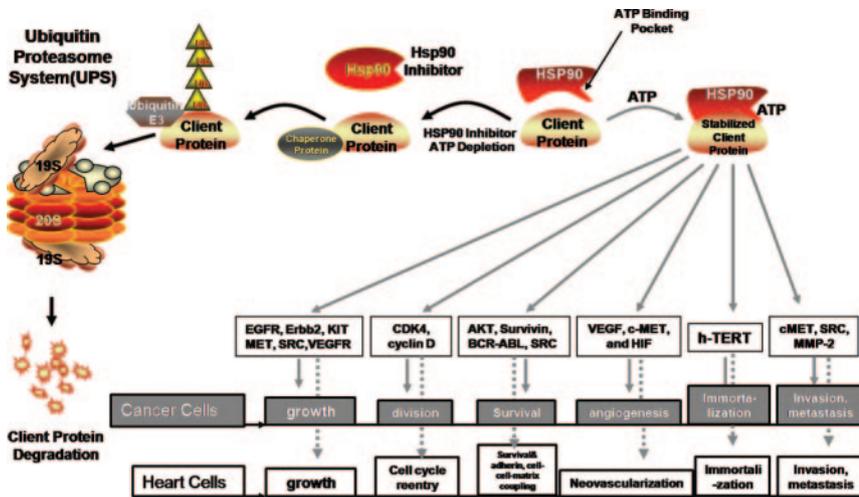
The extrinsic pathway of apoptosis is induced by cytokines of the TNF superfamily acting on death receptors TNF receptor (TNFR)1, TNFR2, TNF-related apoptosis-inducing ligand receptor (TRAILR)1, and TRAILR2.<sup>116</sup> Several agonist antibodies targeting TRAIL receptors are in phase I or II clinical trials for cancer treatment.<sup>117,118</sup>

The effects on the heart of activating the extrinsic pathway are difficult to predict. TNF $\alpha$  overexpression induces cardiomyopathy in mice, raising the concern that chronic activation of some components of this pathway could have adverse effects on the heart. Moreover, recent evidence suggests that fas-associated death domain (FADD) receptor signaling may contribute to stretch-induced cardiomyocyte apoptosis, probably through activating mitochondria-dependent apoptotic signaling.<sup>119</sup> In contrast, TRAIL also increases vascular smooth muscle cell (VSMC) proliferation in vitro, an effect that can be blocked with neutralizing antibodies to TRAIL receptors death receptor (DR)4 and DR1. TRAIL is also required for serum-inducible IGF-1R expression, and antisense IGF-1R inhibits TRAIL-induced VSMC proliferation.<sup>120</sup> Thus, TRAIL may play an important role in promoting atherosclerosis by regulating IGF-1R expression in VSMCs. TRAIL may be one target that suppresses cancer growth while having beneficial effects on cardiovascular disease. To date, no adverse cardiac events have been observed in clinical trial.<sup>121</sup>

### Targeting DNA Repair

Many cytotoxic cancer therapies in current use induce DNA damage, and activate DNA repair mechanisms that allow for cell recovery after sublethal injury. Poly(ADP-ribose) polymerase (PARP-1) is a key enzyme activated by DNA damage that plays a critical prosurvival role in cell cycle arrest and interacts with multiple enzymes involved in DNA repair.<sup>116,122</sup> Inhibitors of PARP-1, including Olaparib and AG-014699 are currently in phase I and II of clinical trials as novel cancer therapeutics and thus far no adverse effects on the heart have been reported.

Activation of PARP enzyme is a crucial step in oxidative stress-induced cardiac and tissue injury.<sup>123</sup> Several studies demonstrate that inhibition of PARP enzyme can efficiently reduce oxidative myocardial damage.<sup>124,125</sup> The underlying mechanism is believed to be through the preservation of the cellular NAD<sup>+</sup> and ATP pools.<sup>126</sup> In spite of the evidence that



**Figure 2. Targeting signaling protein stability via Hsp90 and UP-S.** Hsp90, together with other chaperone proteins, regulates the stability of transmembrane and intracellular signaling proteins involved in cascades. The UP-S regulates function and disposal of proteins, such as erbB family, Raf, and Akt. Disruption of these systems will induce protein misfolding, degradation and activation (such as NF- $\kappa$ B), and suppress cellular function including cell proliferation in cancer cells. Hsp90 and the ubiquitin proteasome system regulate the stability of many of these same proteins in heart cells, with parallel biological consequences. The agents targeting the signaling proteins are indicated in boxes.

PARP-1 inhibition may be beneficial for cardiovascular diseases, a lingering concern is how well PARP inhibitors will be tolerated when combined with cancer therapies that are known to cause genotoxic stress and have been associated with heart failure (eg, anthracyclines and radiation therapy). The mechanism for the late cardiac dysfunction that can occur years after exposure to these therapies is still debated,<sup>127,128</sup> but late consequences of genotoxic stress is likely one contributing mechanism. Moreover, for those patients with specific DNA-repair defects, including those arising in carriers of breast-ovarian cancer (BRCA)1 or BRCA2 mutations, the additional inhibition of PARP mediated DNA repair pathways would likely lead to less effective repair of DNA damage in cardiac tissue as well as in cancer cells.<sup>116</sup> The consequence of this could be early activation of apoptotic cascades with accelerated loss of myocytes. Thus, although development of PARP-1 inhibitors for treatment of cardiovascular disease is exciting, further preclinical research is warranted to assure that these agents can be safely used in conjunction with other forms of cancer therapy.

## Targeting Protein Dynamics With Chaperone and Proteasome Inhibitors

### Chaperone Inhibitors

Heat shock protein (Hsp)90 assists protein folding, stabilizing the ‘client’ protein, and preventing aggregation of denatured proteins<sup>129</sup> (Figure 2). Over the past several years, it has become clear that there are more than 200 Hsp90 client proteins that are involved in almost all cellular processes.<sup>130,131</sup> Many of these client proteins are mutated or overexpressed in cancer.<sup>132,133</sup> Cancer cells particularly depend on intact Hsp90 chaperone function.<sup>134</sup> Inhibition of Hsp90 in cancer cells leads to ubiquitin–proteasome degradation of a large population of oncogenic client proteins,<sup>135</sup> with alterations in multiple steps of carcinogenesis and cancer progression. Hsp90 comprises as much as 4% to 6% of total proteins in tumor cells (versus 1% to 2% in normal cells).<sup>134</sup> The harsh conditions found in the tumor microenvironment including hypoxia, low pH, and metabolic stress may tend to destabilize proteins and augment the requirement for intact Hsp90 function.<sup>136</sup> In cancer cells Hsp90 predominantly exists as multi-chaperone complexes

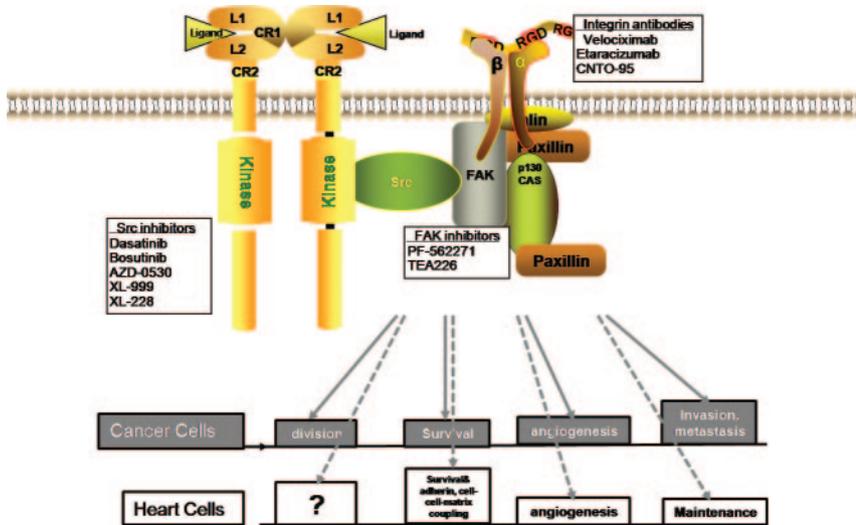
with unusually high affinity for ATP, in contrast to normal cells where Hsp90 is present in an uncomplexed or latent state.<sup>134</sup> This may explain why Hsp90 derived from tumor cells has a 100-fold higher binding affinity for inhibitors such as 17-AAG than Hsp90 isolated from normal cells.<sup>137</sup> Tumor-specific accumulation has been observed for a number of Hsp90 inhibitors, including 17-AAG, 17-DMAG, IPI-504, radicicol derivatives, and purine-scaffold inhibitors.<sup>134</sup> Collectively these observations explain how Hsp90 in tumor is more susceptible to inhibition than that in normal tissues.<sup>136</sup>

Hsp90 does play an important role in the stabilization of erbB2 and other client proteins in the heart.<sup>138</sup> Thus, there is some concern that targeting Hsp90 for suppression of cancer growth may have adverse effects on the heart. However, one positive effect of suppressing Hsp90 is the activation of heat shock factor (Hsf)-1, and induction of a heat shock response including increased expression of Hsp70. Under stressed conditions including cardiac ischemia, this may in fact be cardioprotective.<sup>139</sup> Thus, further work will be necessary to fully understand the cardiac effects of targeting Hsp90 activity on cardiac function and response to stress.

### Proteasome Inhibitors

The ubiquitin–proteasome system (UP-S) is an evolutionarily conserved lysosome-independent cellular protein degradation system that appears essential for growth of some malignant cells.<sup>140</sup> Proper functioning of UP-S is vital to cellular functions including cell-cycle regulation, signaling, differentiation, and DNA repair.<sup>141,142</sup> The UP-S is known to regulate expression of tumor-suppressing, proapoptotic, and oncogenic proteins.<sup>141</sup> UP-S activity requires a sequential process, with multiple steps serving as potential target for therapeutic development. Preclinical studies have shown that proteasome disruption inhibits proliferation, induces apoptosis, reverses chemoresistance,<sup>143</sup> and enhances chemotherapy and radiation. Several proteasome inhibitors have been evaluated in clinical trials, and more are in development.

Recent investigations of the proteasome pathway indicate that the UP-S is critical for maintenance of cardiac structure and function, and that altered proteasome function is associated with cardiac pathophysiology.<sup>141</sup> Marked accumulation



**Figure 3. Integrins and the downstream signaling proteins of focal adhesions are targeted in cancer, and serve important functions in the heart.** Integrin signaling is involved in cell division, survival, angiogenesis, invasion, and metastasis in neoplasms and provides targets amenable to therapeutic interventions in cancer therapy. The targets are present in heart cells such as myocytes, although their functions are incompletely understood. The agents targeting the signaling proteins are indicated in boxes.

of ubiquitinated proteins occurs in the failing heart, suggesting impaired UP-S activity.<sup>144</sup> Loss of the activity of the 20S and/or 26S proteasome in parallel with increased levels of oxidized and ubiquitinated proteins have been observed in rat hearts after ischemia/reperfusion injury.<sup>145</sup> Proteasome dysfunction in cardiac hypertrophy may contribute to the transition to heart failure through the accumulation of proapoptotic proteins.

Bortezomib (Velcade, PS-341), a boronic acid derivative, was the first proteasome inhibitor to be developed successfully as a cancer therapeutic. Bortezomib blocks proliferation and induces apoptosis of plasma cells.<sup>146</sup> It also inhibits the proteasomal degradation of I $\kappa$ B- $\alpha$  and thereby suppresses the antiapoptotic and proinflammatory transcription factor NF- $\kappa$ B, leading to enhancement of chemotherapy sensitivity. Bortezomib was recently approved by the FDA for initial treatment of patients with multiple myeloma based on its effects on plasma cell proliferation. The side effects reported include asthenia, peripheral neuropathy, gastrointestinal symptoms, and anorexia. A few reports of cardiac failure occurring in patients during treatment with bortezomib<sup>147–149</sup> suggest that under some circumstances inactivation of the cardiac proteasome can result in adverse effects on the heart. These observations suggest that further work is warranted to understand the consequences of inhibiting this pathway in the heart, considering that other proteasome inhibitors are in early phase trials including CEP-18.770, RP-171, and NPI-0052.<sup>150</sup>

### Extracellular Matrix/Integrin System and Focal Adhesion Complexes

The target of Velociximab ( $\alpha$ 5 $\beta$ 1) is expressed during cardiac embryonic development.<sup>151</sup>  $\beta$ 1 integrin is the main  $\beta$  isoform expressed in the adult tissue and can modulate several downstream signaling pathways, such as focal adhesion kinase (FAK), sarcoma (Src), p130CAS, and Ras.

Integrins are heterodimeric membrane receptors that regulate cellular adhesion to the extracellular matrix<sup>152</sup> (Figure 3). Integrins are involved in proliferation,<sup>153</sup> survival,<sup>154</sup> migration,<sup>155</sup> and angiogenesis<sup>156</sup> by aggregating with adaptor

proteins and kinases, such as paxillin, Src, FAK, and other components of focal adhesion complexes.<sup>157</sup> Largely because of their role in angiogenesis, integrins have been considered as a target for immunotherapy. Velociximab and etaracizumab, are both humanized monoclonal antibodies in early phase clinical trials. Like other angiogenesis inhibitors, hypertension has been observed in conjunction with integrin targeted therapy. In addition, myocardial ischemia has been observed in a small fraction of patients.<sup>158–160</sup>

Integrins regulate pathological conditions including hypertrophy induced by either mechanical stress or by growth stimuli such as phenylephrine and endothelin-1. Restricted deletion of  $\beta$ 1 integrin in myocytes leads to myocardial fibrosis and development of spontaneous dilated cardiomyopathy in 6 month old mice, as well as an exaggerated response to pressure overload without evidence of cell death.<sup>161</sup>  $\beta$ 1 dominant negative transgenic mice die perinatally and their hearts display extensive fibrotic replacement.<sup>162</sup> Loss of  $\beta$ 5 in myocytes results in a milder phenotype at baseline that can be exacerbated by transverse aortic constriction.<sup>163</sup> Mice with gain-of-function mutation in  $\alpha$ 5 integrin develop fibrotic changes, with decreased expression of connectin-43, loss of functional gap junctions, and abnormal intercalated disks.<sup>164</sup> This is associated with atrial fibrillation, as well as bradycardia, systolic dysfunction, and perinatal mortality.<sup>164,165</sup>

Src and FAK are activated by integrins, and they are ubiquitously expressed and localize to the cell-matrix junction. Alterations in Src/FAK signaling has been associated with several tumors, including breast, colon, pancreatic, ovarian, cervical, kidney, lung, and melanoma.<sup>166</sup> Their overexpression is often associated with higher invasiveness and metastasis formation.<sup>166,167</sup> Several Src inhibitors are currently in clinical trials, such as Dasatinib, Bosutinib, AZD-0530, XL-999, and XL-228. Dasatinib has been approved by FDA with promising results.<sup>168</sup> Several FAK inhibitors are currently in clinical trials, such as PF-00562271.<sup>169</sup>

In the heart, FAK is activated in response to pressure overload,<sup>170</sup> mechanical stretch and deformation of adhesion

plaques,<sup>171</sup> atrial natriuretic factor,<sup>171</sup> angiotensin II,<sup>172</sup> and endothelin I.<sup>173</sup> FAK also plays an important role in hypertrophy and cardiomyocyte survival. Mice with cardiac deletion of FAK are viable, but under pressure overload develop a dilated cardiomyopathy.<sup>174</sup> These observations suggest that inhibition of Src and FAK would lead to cardiac effects via alterations of cell–cell and cell–matrix interactions. So far symptomatic cardiac dysfunction has only been observed in 2% of patients treated with Dasatinib.<sup>168</sup> The important role of focal adhesions in maintaining cardiac structure and function suggests that continuous monitoring of the heart in these patients.

### Concluding Remarks

The rapid pace of biological discovery in cancer cell biology has led to an expanding array of strategies moving rapidly into clinical trials for the treatment of cancers refractory to established therapies. Heart cells including myocytes, microvascular-endothelial cells, vascular smooth muscle cells and fibroblasts share signaling pathways and biological processes with cancer cells. Understanding the molecular mechanisms and function of those targets in the cardiovascular system and other vital tissues becomes an important step in the development of these therapies.

One difference between malignant and nonmalignant cells and tissues including the heart is that the signaling activity will vary with time. For many potential cancer targets, it is likely that signaling activity in the heart is increased during times of stress. Defining the timing of cardiac activity may therefore be a critical determinant of the relative size of the therapeutic window success of some of these novel therapies.

Obviously further basic investigation is necessary, and should be encouraged in laboratories with an interest in the cardiovascular role of these cancer targets. Understanding the mechanism for effects of drugs on cardiovascular function may lead to strategies that can prevent adverse events. Mechanistic insights should also help to determine timing of drug administration, thereby minimizing the severity of cardiotoxicity. Furthermore, it can become a discovery platform for the mechanistic regulation of cardiovascular system. Involving clinical cardiovascular investigators in early and late phases of clinical oncology trials will be an important strategy to improve outcomes for cancer patients. More detailed patient screening for subclinical cardiovascular dysfunction is warranted in patients being considered for treatment with novel agents that have potential cardiac toxicity. This could potentially involve biomarkers and screening for genetic predisposition to cardiovascular disease. Cardiovascular outcomes beyond the usual QT interval and left ventricular ejection fraction may be warranted in the case of some therapies. Fostering collaborations between cardiology and oncology investigators across the spectrum of basic to clinical research will be critical in this effort.

### Acknowledgments

We thank Drs Carlos Arteaga, David Slosky, Dan Lenihan, and Michael Freeman for helpful discussions.

### Sources of Funding

This work was funded by an American Heart Association Beginning Grant-in-Aid, Amyloidosis Foundation Research Grant to Xuyang Peng, and American Heart Association Established Investigator Award and NIH HL068144 to Douglas B. Sawyer, pilot project from SPORE in Breast Cancer 5 P50 CA098131 to Vanderbilt University.

### Disclosures

D.B.S. has received grant support from Genentech, Inc, Roche, Inc, and Acorda Therapeutics, Inc. DBS has been a consultant for Oncocyte, Millenium Pharmaceuticals, and Regeneron.

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Xuyang Peng, Laura Pentassuglia and Douglas B. Sawyer

*Circ Res.* 2010;106:1022-1034

doi: 10.1161/CIRCRESAHA.109.211276

*Circulation Research* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231

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Print ISSN: 0009-7330. Online ISSN: 1524-4571

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