Cardiotoxicity after anticancer treatment

Perik, Patrick Jozef

2

The dilemma of the strive for apoptosis in oncology: Mind the heart

Patrick J. Perik [1,2]
Elisabeth G.E. de Vries [2]
Jourik A. Gietema [2]
Winette T.A. van der Graaf [2]
Dirk Th. Sleijfer [2]
Albert J.H. Suurmeijer [3]
D.J. van Veldhuisen [1]

[1] Department of Cardiology
[2] Department of Medical Oncology
[3] Department of Pathology
University Medical Center Groningen, The Netherlands

Critical Reviews in Oncology and Hematology 2005;53:101-113

ABSTRACT

In recent years, apoptosis has increasingly drawn the attention of both oncologists and cardiologists alike. Anticancer treatment is possible by induction of apoptosis in cancer cells, and targeted anticancer drugs are being developed to promote this. However, since these drugs usually are not selective for malignant cells, side effects on non-cancerous tissue, such as the myocardium must be anticipated. Since apoptosis is a pathophysiological mechanism in cardiac diseases leading to heart failure, cardiologists in contrast to oncologists, aim at preventing apoptosis in the heart. The purpose of this review is to describe new insights in mechanisms of cardiomyocyte apoptosis. In addition to the mitochondrial and death receptor apoptotic pathways, apoptosis through lack or inhibition of growth factor receptor-mediated signalling is discussed. Exploration of the apoptotic pathways in the heart can contribute to the safer use of new anticancer drugs and to the development of new therapies for heart failure.

INTRODUCTION

Cardiac toxicity is a serious adverse effect that can accompany the use of several chemotherapeutic agents. Anthracyclines in particular are related to an increased risk of cardiac dysfunction, which is mostly due to cardiomyopathic changes, causing impaired myocardial contractile function. Impaired myocardial contractile function can reflect a reduction of the number of functional myocytes, decreased contractility of viable myocytes or both. Myocyte loss can be caused by autophagic cell death,[1] necrosis and apoptosis. Autophagy consists of sequestration of cellular material into double membrane vacuoles that fuse with lysosomes, which are subsequently degraded by lysosomal proteases.[2] Necrosis, or better oncosis, is a passive process of cell death that occurs when a cell is deprived of oxygen. In contrast, apoptosis is an active, energy consuming process of controlled cellular and organellular destruction and has been described as an important pathophysiological factor in chronic heart failure (CHF).[3-5] Apoptotic pathways are extensively studied to develop new strategies for inducing cell death in cancer cells. New drugs are being developed that specifically target key components involved in tumour cell apoptosis. However, a major concern is that these new compounds increase apoptosis in organs, such as the heart. Although the role of apoptosis in the pathophysiology of CHF is still controversial, the discovery of previously unknown apoptotic pathways in cancer research may place cardiac apoptosis in another perspective. The aim of this paper is to describe new insights in the molecular pathways underlying cardiomyocyte apoptosis. Inhibition of growth factor receptor signalling, which is currently widely investigated in anti-tumour treatment, may also induce apoptosis in the heart. Growth factor receptor signalling pathways will therefore be emphasized here. In addition, the relevance of these apoptotic pathways for clinical practice will be reviewed.

MECHANISMS OF APOPTOSIS

Apoptosis can be induced by stimuli activating the intrinsic pathway (mitochondrial pathway), the extrinsic pathway (death receptor pathway), or both (Figure 1). In addition, growth factors and their receptors, e.g. the epidermal growth factor receptor family (EGFR family), promote cell growth and survival, thereby inhibiting apoptosis. Absence, deficiency or inhibition of growth factor receptor signalling causes apoptosis to increase, for instance by the monoclonal antibody against human epidermal growth factor receptor 2 (HER2), trastuzumab. Apoptosis can be initiated by intracellular and extracellular triggers, including DNA damage, a defective cell cycle, hypoxia, detachment of cells from their surrounding tissue and loss of trophic signalling. Caspases play an important downstream role in the intracellular apoptotic signalling cascade. These proteases form a family, consisting of three functional groups based on their substrate

specificities.[6] Caspase 2, 3 and 7 cleave specific structural proteins and key intracellular DNA repair enzymes (e.g. actin, Poly ADP ribose polymerase). These proteins function as executors of apoptosis and are activated by initiator caspases (Figure 1). The remaining group of caspases, caspase 1, 4, 5, 11, 12 and 13, are merely involved in transduction of apoptotic signals.
Oxidative cardiac stress primarily induces "mitochondrial apoptosis".[7,8] Activation of receptors belonging to the tumour necrosis factor receptor (TNF-R) superfamily, can also induce apoptosis. However, mitochondrial apoptosis is considered to be the main pathway for cardiac apoptosis, since inhibitors of the death receptor pathways in the heart are relatively overexpressed.[9] Deficient growth factor receptor signalling can also lead to apoptosis via the intrinsic and extrinsic pathway (Figure 1 and 5).

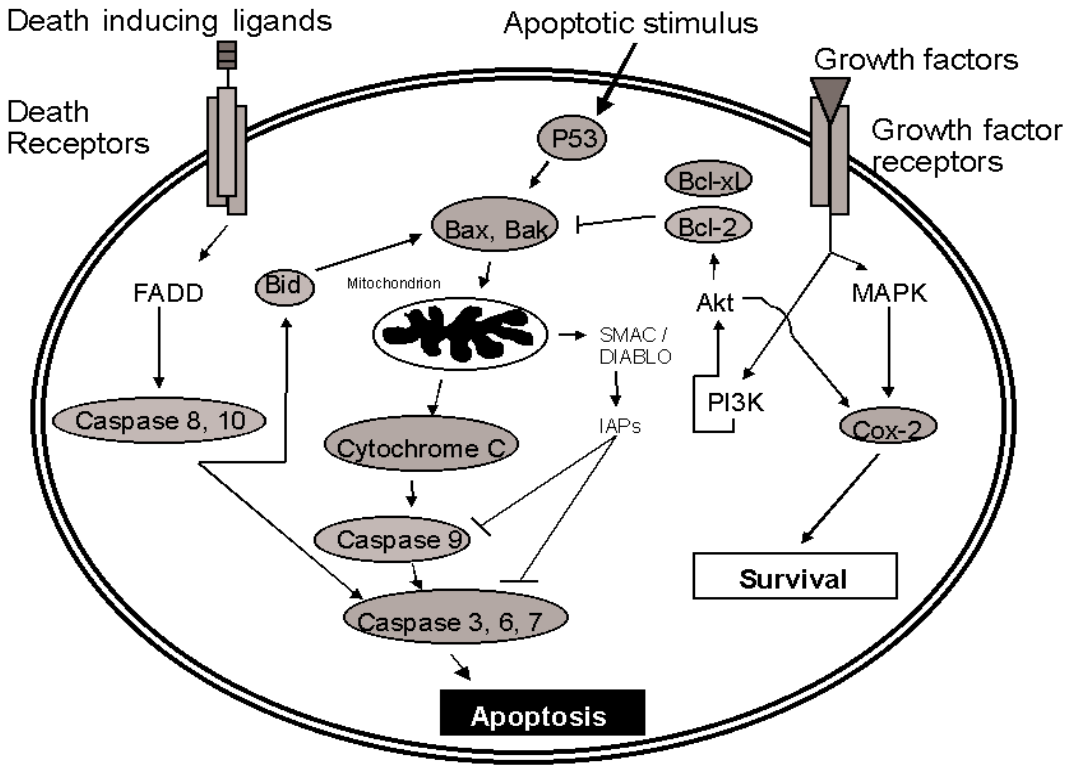

Figure 1. Schematic overview of cardiomyocyte apoptotic pathways. Death receptor pathway: intracellular signaling cascade upon ligand binding. Death receptors are TNF-R1 and 2, Fas, DR4 and DR5. Death-inducing ligands are TNFα, Fas ligand and TRAIL. Mitochondrial pathway is activated following an apoptotic stimulus mediated by p53. Growth factor receptor signaling occurs upon binding of a growth factor to the specific growth factor receptor. HER1/EGFR can bind EGF, transforming growth factor-α, β-cellulin, HB-EGF, amphiregulin and epiregulin. No cognate ligand has been discovered for HER2. Neuregulin can activate both HER3 and HER4 subtypes. HGF binds to HGFR.

Intrinsic apoptosis

The mitochondrion is the pivotal site for the intrinsic apoptotic pathway. The common activator for this route is cytochrome c, which is released from the intermitochondrial space (Figure 1). Once released in the cytosol, cytochrome c

promotes pro-caspase 9 cleavage into its active form.[10] Intrinsic apoptosis is regulated by the *Bcl-2*-gene family of pro- and anti-apoptotic factors, which consists of at least 18 proteins.[11] In cardiomyocytes, Bcl-2 inhibits mitochondrion-related apoptosis in isolated neonatal ventricular muscle cells.[12] However, *Bcl-2* knock-out mice show no increased cardiomyocyte apoptosis, suggesting that *Bcl-2* is not indispensable for cardiomyocyte apoptosis inhibition.[13] Interestingly, the recently discovered pro-apoptotic *Bcl-2* family member, *Bcl-2* interacting protein (BID), links the intrinsic and extrinsic apoptotic pathways (Figure 1).[14]
The tumour suppressor gene *p53* also has apoptosis regulating properties (Figure 1). *P53* mutations can down-regulate p53 expression, which induces tumour growth through inadequate suppression of cellular proliferation by decreased apoptosis.[15] However, p53 also plays a role in cardiomyocyte apoptosis. Elevated p53 protein levels in cultured rat cardiomyocytes after hypoxia are related to increased apoptosis.[16] P53 is however not indispensable for cardiomyocyte apoptosis, as p53 is involved in mitochondrion-related apoptosis and not in apoptosis by death receptors.[17] Increased Fas expression is linked to p53, suggesting that p53 provides the link between the intrinsic and extrinsic apoptotic pathways.[18]

Extrinsic apoptosis
The extrinsic pathway is activated upon ligand binding to its specific membrane-bound death receptor (Figure 1). Death receptors belong to the TNF superfamily of receptor-proteins, which comprises almost 20 members. Increased levels of apoptosis-related cytokines are present in acute coronary syndromes and CHF.[19] In left ventricles of explanted hearts from patients with terminal CHF, caspase 9, indicative of mitochondrial apoptosis, and caspase 8 were activated, pointing to death receptor-mediated apoptosis.[20]
Death receptors form homotrimers upon activation, which subsequently bind intracellular signalling molecules, resulting in formation of the death-inducing signalling complex (DISC), which contains intracellular apoptosis mediating signal molecules, e.g. TNF-R-associated death domain protein (TRADD), Fas-associated death domain (FADD) and TNF-R-associated factor (TRAF). The DISC plays a central role in extrinsic apoptosis by activating the initiator caspases 8 and 10.[21] As in mitochondrial apoptosis, this is followed by activation of the effector caspases, which start apoptotic processes within the cell.
Next, TNFα and its receptors, Fas ligand and Fas, followed by TNF-related apoptosis-inducing ligand (TRAIL) will be discussed concerning their role in extrinsic cardiac apoptosis.

TNFα Various cell types produce TNFα, including leukocytes, endothelial cells, cardiac fibroblasts and cardiomyocytes.[22] TNFα plays a role in cell death processes (Figure 2), cellular growth and inflammation, through activation of its receptors, p55/TNF-R1 and p75/TNF-R2.[23] Due to severe side effects, systemic recombinant human TNFα administration is not applied in anti-tumour treatment is. In the

cardiac apoptosis however, endogenous TNFα plays an important role. In 50 patients with acute myocardial infarction, serum TNFα levels were elevated, and correlated with infarct size. Peak TNFα levels were found 84 hours after admission.[24] In CHF patients, cross-sectional measurements revealed 10-fold higher TNF levels, compared to healthy controls.[25,26] Serum TNFα levels also correlated with CHF severity.[27] This suggests that serum TNFα in the course of CHF may be a valuable marker for disease severity. However, it has limited specificity, since it is also increased in diseases, such as Crohn's disease and rheumatoid arthritis.

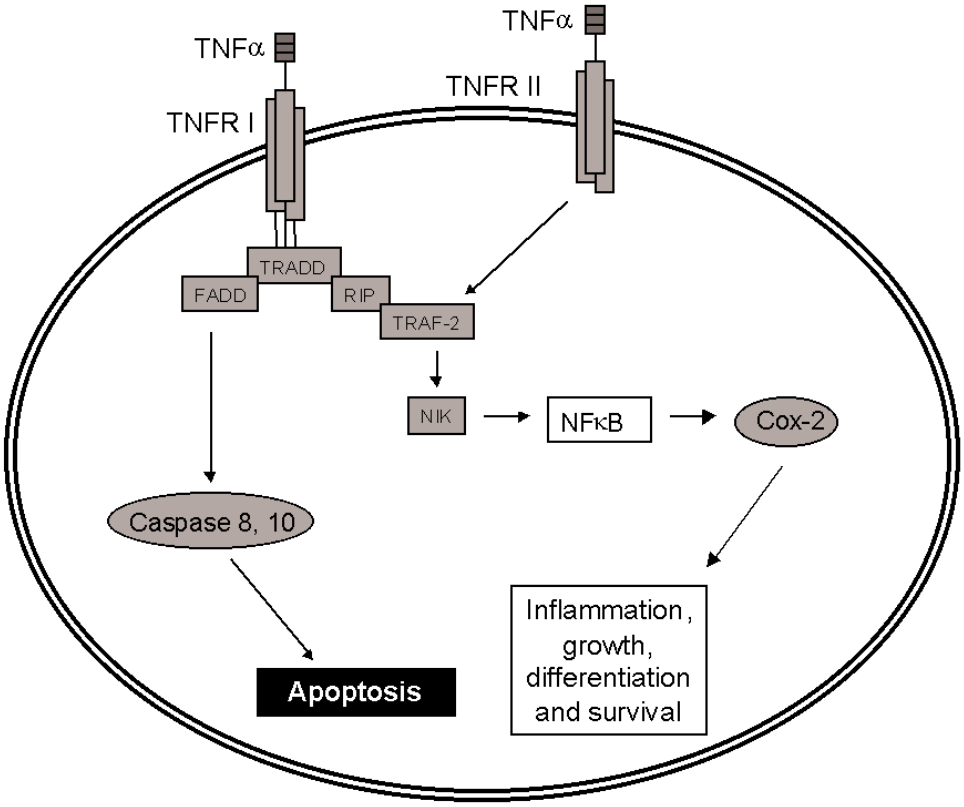

Figure 2. Schematic overview of molecular pathways for TNFα-induced cardiomyocyte apoptosis through activation of its cognate receptor TNF-R1 and 2. TNFα exerts pro-apoptotic effects via activation of the caspase cascade, and pro-survival effects through activation of NFκB, and COX-2. NIK, nuclear factor nuclear factor κB-inducing kinase; NFκB, nuclear factor κB; RIP, receptor-interacting protein.

Decreased TNFα production may potentially limit apoptotic cardiomyocyte loss. Pentoxifylline, an immunomodulatory agent, with some activity in severe refractory intermittent claudication, inhibits TNFα. Small clinical studies in CHF showed beneficial effects on left ventricular function and signs and symptoms of CHF, with pentoxifylline orally.[28,29] Two other drugs, etanercept (a TNF-R fusion protein) and infliximab (a monoclonal antibody with high affinity and specificity for TNFα) inhibit TNFα signalling, by preventing ligand-receptor coupling. Both agents are used for active rheumatoid arthritis and infliximab also for Crohn's disease. Three large randomized trials in CHF patients, performed after promising small pilot studies,[30-32] revealed no improvement in death or hospitalization rate of

etanercept or infliximab however. In one study (RENAISSANCE), hazard ratios even tended to increase by etanercept.[33] It is striking that doses as high as for Crohn's disease were used. This may have clouded the observation, as one would expect lower doses to be adequate for CHF.[33] Nevertheless, these trials suggest limited value of anti-TNF therapy for CHF.

Fas Ligand The type I transmembrane receptor Fas (APO-1) can induce apoptosis upon binding of Fas Ligand (APO-1L) presented by an effector cell (Figure 3).[34] The extracellular domain of Fas and Fas ligand can be shed into the circulation.[35] As with TNFα, systemic adminis tration of anti-Fas antibodies or Fas ligand for the treatment of human malignancies appears not to be feasible, due to severe liver damage observed in mice.[36,37]

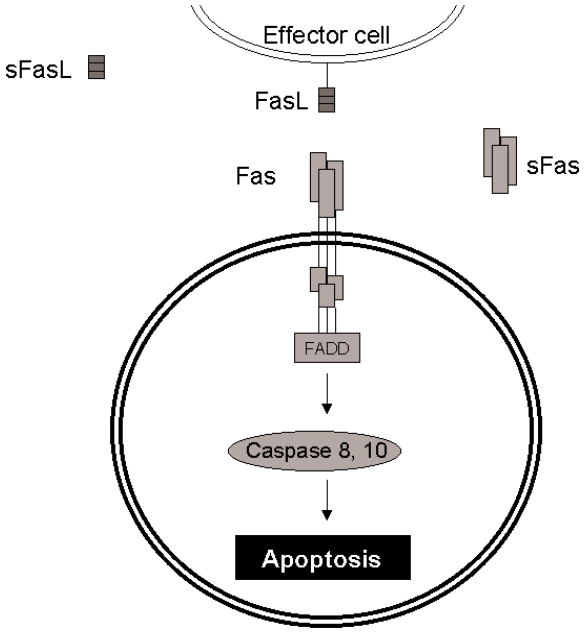

Figure 3. Schematic overview of Fas – Fas ligand-induced cardiomyocyte apoptosis. Fas binds Fas ligand, a transmembrane protein attached to an effector cell, mostly cytotoxic T-cells. The intracellular death domain subsequently binds FADD, after which apoptosis is induced through caspase activation.

Fas is abundantly expressed in animal myocardial tissue and cardiac myocytes.[38] In vitro data and animal studies showed that Fas is involved in cardiomyocyte apoptosis due to ischemia, and CHF.[39-41] In ischemia-based CHF patients, Fas expression in left ventricular biopsies obtained before coronary artery bypass grafting was increased in fibrotic areas. However, these areas showed no increased apoptosis.[42]

Serum soluble Fas levels correlate with functional class (New York Heart Association) of CHF, with highest levels in patients with NYHA class IV.[43,44] Serum soluble Fas levels in 96 NYHA class II-IV CHF patients, was an independent predictor for survival.[45] However, there was no correlation between serum levels of soluble Fas and natriuretic peptides (atrial natriuretic peptide (ANP) and brain-derived natriuretic peptide (BNP)).[45]

Inhibition of Fas-mediated cardiomyocyte apoptosis may lead to new options in the treatment of CHF. β-adrenergic blockade in rabbits with carvedilol 1 mg/kg intravenously, inhibited Fas-mediated apoptosis.[39] In patients with dilated

cardiomyopathy, administration of growth hormone or pentoxifylline improved CHF by down-regulating serum soluble Fas and TNFα levels.[29,46]
Recently, controversy was introduced regarding the pro-apoptotic role for Fas in the heart. Fas signalling was required for the compensatory hypertrophic response following left ventricular pressure overload in lymphoproliferative mice, which lack functional Fas.[47] This is in accordance with the observation that Fas induced hypertrophic transcription factor activator protein-1 in cultured cardiomyocytes.[38]
In conclusion, Fas plays a complex role in the myocardium and may be required, in concert with other factors, as an adaptive compensatory mechanism to compensate cardiac stress.

TRAIL The type II transmembrane protein TRAIL (APO-2L) has five receptors (Figure 4).[48] Only two can transmit the signal intracellularly, namely death receptors DR-4 (TRAIL-R1),[49,50] and DR-5 (TRAIL-R2)[51] DcR1 (TRAIL-R3)[51] and DcR2 (TRAIL-R4),[52] are decoy receptors, lacking a functional death domain. The fifth TRAIL-binding receptor is the soluble osteoprotegerin, a regulator of osteoclastogenesis. Contrasting TNFα, anti-Fas antibodies and Fas ligand, recombinant hu man TRAIL is a very promising agent for systemic use in anticancer treatment. Following animal studies, in which anti-tumour effects and chemopotentiating effects of systemic TRAIL were observed, phase I studies with TRAIL will soon start. Phase I studies with an agonistic TRAIL-DR-4 antibody are ongoing.

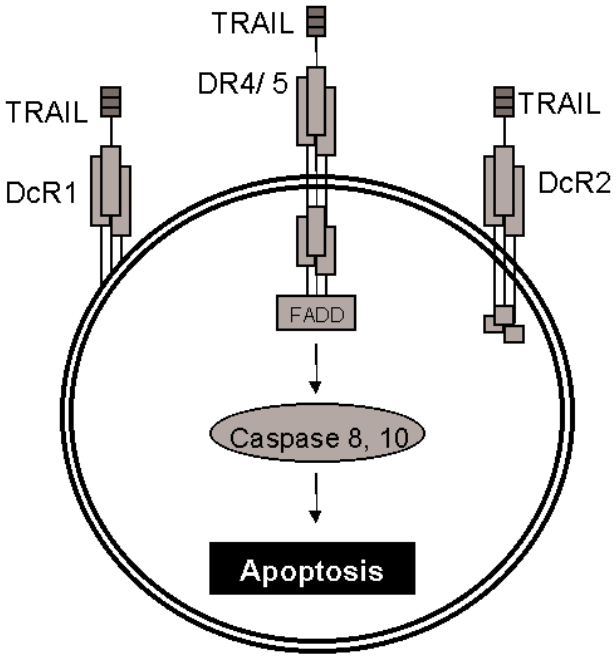

Figure 4. Schematic overview of TRAIL-induced cardio-myocyte apoptosis. TRAIL induces apoptosis via DR4 and DR5 activation. DcR1 and DcR2 lack an intracellular signaling domain. They are capable of binding TRAIL competitively, thereby inhibiting the function of TRAIL.

We showed that DR4, DR5, DcR1 and TRAIL are present in chimpanzee and human cardiac myocytes.[53] Furthermore, Western blot analysis of concentrated transudates from explanted rat and murine hearts subjected to ischemia/reperfusion (Langendorff model) revealed that TRAIL is released into extracellular fluid, early after reperfusion.[54] Accordingly, cardiomyocyte TRAIL expression was increased

in explanted rat and murine Langendorff hearts.[54] Microarray experiments of peripheral blood mononuclear cell of CHF patients showed that, several genes encoding for TNF-related cytokines, including DcR2 but not DcR1, were up-regulated, compared to healthy individuals. This was confirmed by quantitative RT-PCR for TRAIL and TNFα.[55] TRAIL-mediated apoptosis may therefore be involved in cardiomyocyte apoptosis in CHF.
Exposure of human umbilical vein endothelial cells (HUVECs) to TRAIL, on the other hand, resulted in inhibits apoptosis,[56] and increases production of the crucial vascular regulator nitric oxide (NO).[57] This may be caused by the relative overexpression of DcR1 in HUVECs.[58] Since TRAIL is known as an apoptosis inducer, the observation that TRAIL inhibits endothelial cell apoptosis, is surprising, and insinuates that the physiologic role of TRAIL may be tissue specific and more complex than initially assumed.

Apoptosis by defective growth factor receptor signalling

Decreased growth factor receptor-mediated signalling can also lead to apoptosis through loss of cell survival promoting stimuli. In the following section, the role of the human epidermal growth factor receptors (HER) 1 to 4 in the heart will be discussed, followed by the cardiac role for hepatocyte growth factor (HGF) and its receptor (HGFR). Finally, COX-2 will be discussed as the pivotal enzyme involved in growth factor receptor-mediated intracellular signalling. Growth factor receptor signalling plays an important role in embryonic development of several tissue types, including the heart. To date, the EGFR family is the best known and most extensively studied type of growth factor receptors. This family has four members, HER1 (ErbB-1 / EGFR), HER2/*neu* (ErbB-2), HER3 (ErbB-3) and HER4 (ErbB-4) (Figure 5).

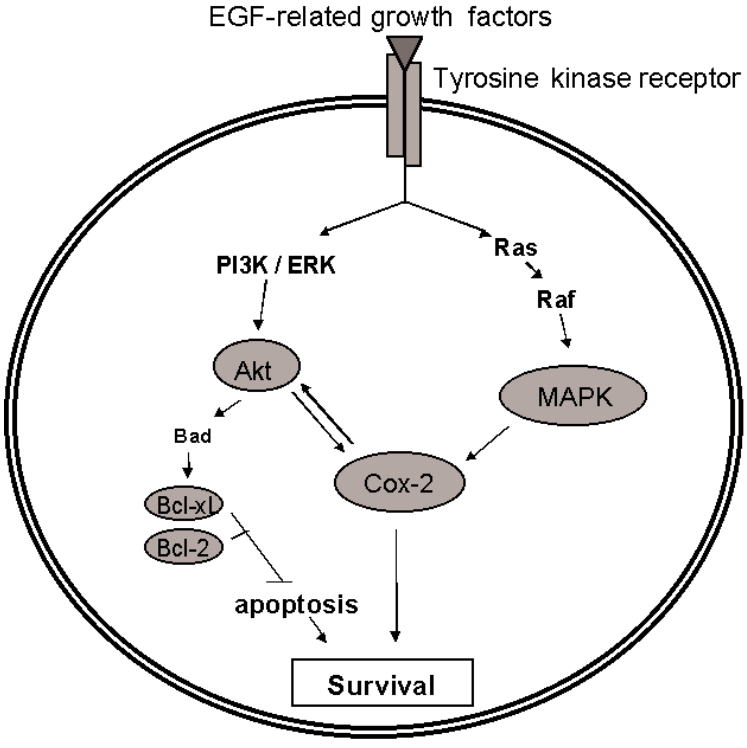

Figure 5. Schematic overview of growth factor receptor-mediated cardiomyocyte apoptosis. After growth factor binding to the cognate tyrosine kinase receptor, receptor dimerization takes place. Cardiomyocyte survival and apoptosis inhibition is promoted through activation of the PI3K/ERK – Akt pathway and the MAPK route. COX-2 plays a pivotal role in growth factor receptor signaling. Ras and Raf are cell growth regulatory proteins.

All are type I transmembrane receptor tyrosine kinases and share functional homology with EGFR.[59] Receptor dimerisation, which occurs upon ligand binding, is required for intracellular signal transmission. In the process of dimerisation, both homo- and heterodimerization with other HER family members can occur, depending on the receptors present and the ligand involved.[60] Each possible dimerized receptor complex activates a different intracellular signalling pathway, which thus greatly broadens the signalling diversity. Signal transducer and activator of transcription (STAT) factors and mitogen activated protein kinase (MAPK) are up-regulated following HER activation, which results in cellular growth, differentiation and survival.[61] This trophic signalling is required for normal development and physiology of several tissues. In the heart, growth factor receptor signalling is required for the process of trabecularization and formation of the cardiac valvular system.[62] In addition, all four HER subtypes are involved in angiogenesis and formation of endothelium, which was demonstrated in HUVECs.[63]

HER1 (erbB-1/ EGFR) is the archetype of the EGFR family of type I transmembrane growth promoting receptors. HER1 transmits signals provided by EGF, transforming growth factor α (TGFα), betacellulin, heparin-binding EGF-like growth factor (HB-EGF), amphiregulin, epiregulin and epigen (for review see [59]). HER1 is involved in embryonic development of several tissue types. This was shown in HER1 knock-out mice which, if surviving, exhibit multiple severe epithelial and cerebrovascular abnormalities.[64] HER1 is frequently overexpressed in human cancers and is already extensively explored as a target for anti-tumor therapy. The HER1 antibody cetuximab was registered February 2004 as anti-tumor drug in the treatment of metastatic colon cancer after failure of irinotecan treatment.

After HER1 was detected in cultured embryonic chicken heart muscle cells,[65] it was found to be involved in valvulogenesis. HER1 knock-out mice show marked thickening of semilunar valves with increased numbers of mesenchymal cells, compared to mice capable of expressing HER1, while atrioventricular valves and the interventricular septum of the HER1 knock-out mice are normal.[66] This suggests that HER1 is specifically required for semilunar valvulogenesis. In addition, HER1 stimulates proliferation of cardiac myocytes, but inhibits their differentiation.[67]

A key factor in the development of left ventricular hypertrophy (LVH) is the angiotensin II type 1 receptor and activation of this receptor induces tyrosine phosphorylation by HER1 “trans” activation.[68,69] HB-EGF is related to hypertrophic growth responses, as its mRNA expression is enhanced in isolated myocytes from explanted spontaneously hypertensive rat hearts.[70]

HER1 plays a role in cardiac remodeling after myocardial infarction. In rats, 6 weeks after induction of myocardial infarction, HB-EGF mRNA and protein are present in infarcted areas with myocardial tissue replacement by extracellular

matrix and interstitial fibrosis.[71] Additionally, HER1 has cardioprotective effects.[72] Administration of the HER1-specific tyrosine kinase inhibitor, AG 1478, 25 mg/kg intraperitoneally, to mice just before exposure to inter-male fighting (60 min), increases serum cardiac isoform lactate dehydrogenase 1 and creatine kinase, 3 hours after inter-male fighting, compared to non-fighting and untreated mice. In mice, 0.25 mg/kg exogenous EGF intraperitoneally, 20 minutes before exposure to restraint and cold (4°C) for 1 hour, results in less lactate dehydrogenase 1 and creatine kinase serum activity, compared to non-EGF-treated animals, 7 hours later. Simultaneous administration of AG1478 completely abolishes this EGF effect.[72]

HER2 signalling is required for normal embryonic development. No specific ligand has been discovered for this EGFR subtype. Nevertheless, HER2 is the preferred heterodimerization partner for the other HERs, with a pivotal role in HER cross-talk.[60] Upon receptor-ligand binding, the HER2-HER3 heterodimer is the preferred couple, with highest signalling potential. Next to its role in the heart (discussed below) HER-related signalling is important for growth and development of neural tissue, and skeletal muscle.[73] To date, HER2 is predominantly known for its oncogenic role. It is amplified and/or overexpressed in a variety of human tumours.[74] Overexpression occurs in 25-30% of breast cancers, and adversely affects patient prognosis and survival.[75] HER2 can be targeted by trastuzumab (Herceptin®).[76] In a phase III trial, comparing efficacy of standard chemotherapy plus or minus trastuzumab in HER2 overexpressing metastatic breast cancer patients, an increased incidence of CHF was observed in the trastuzumab group, underlining the myocardial role for HER2. Since trastuzumab cardiotoxicity has particularly been encountered with concurrent anthracycline chemotherapy, a synergistic effect of the combination resulting in cardiac toxicity is probable.[77] In vitro, trastuzumab induces phosphorylation of cardiomyocyte HER2.[78] Furthermore, trastuzumab increased myofibrillar disarray in anthracycline treated rat cardiomyocytes. This indicates that trastuzumab enhances the susceptibility of cardiomyocytes to anthracyclines.[79] Additionally, alternative mechanisms for trastuzumab cardiotoxicity have been suggested. For instance, altered excitation-contraction coupling in cultured rabbit cardiomyocytes occurs after EGF administration.[80] Increased cardiomyocyte apoptosis, through loss of trophic signalling by HER2 may also play a role.
In embryonic wild-type mice, HER2 is immunohistochemically present in myocardial and endocardial cells.[81,82] Cardiomyocyte HER2 expression is mostly restricted to the T-tubular network, indicating a non-random cardiac distribution pattern.[83] It is therefore likely that HER2 regulates circumscript processes in cardiac physiology. The first evidence indicative of HER2 involvement in the heart, stems from HER-deficient, and neuregulin deficient mice, which die early (before day 10.5) in embryonic development.[81,84-87] Neuregulin deficient mice exhibit neural crest deformities alongside cardiac abnormalities, which consist of poor trabecularization and underdeveloped endocardial cushion mesenchyme.[87]

Neuregulin treatment can rescue cardiovascular development by restoring mesenchyme formation. This was demonstrated in explanted atrioventricular canal tissue from mutated mice lacking the gene coding for glycosaminoglycan hyaluronan, which is necessary for cardiac septal and valvular formation.[82,86] HER2 gene deletion in mice results in complete lack of trabecularization, but only minor endocardial cushion abnormalities.[81] In addition, mice with a mutation that inactivates the HER2 tyrosine kinase domain, die at midgestation and show similar embryonic defects as HER2 knock-out mice.[88]

Specific myocardial HER2 cDNA induction, rescues genetically altered HER2 deficient mice to survive beyond birth.[89,90] Neurologic abnormalities are however not prevented. HER2 also appears to be required beyond embryonic development, since HER2 (and HER4) mRNA is present in adult murine myocardium.[62]

Neuregulin administration to cultured embryonic rat ventricular myocytes decreases apoptotic rates (TUNEL).[62] Furthermore, neuregulin induces a hypertrophic growth response with increased ANP mRNA levels in isolated rat heart muscle cells. Since ANP is known to induce apoptosis,[91] it is remarkable that neuregulin treatment inhibits apoptosis. Again, this suggests that HER2 is required for cardiac physiology, e.g. cardiomyocyte survival. HER2 is crucial for the prevention of dilated cardiomyopathy. This was demonstrated in conditional mutant mice with a cardiac-restricted HER2 gene deletion. These mice were born at normal Mendelian frequencies and showed no overt phenotypic abnormalities at birth. However, shortly after birth they developed dilated cardiomyopathy, with severely attenuated myocardial contractility.[83,92] Strikingly, TUNEL showed no increased myocardial apoptosis.[83] A sensitive PCR DNA-fragmentation assay however, showed increased cardiac apoptosis in cardiac-restricted HER2 knock-out mice.[92] Subsequent adenoviral introduction of Bcl-xL expression in newborn conditional mutated mice, resulted in partial rescue of both chamber dilation and contractility, with decreased left ventricular end diastolic diameter and increased fractional shortening.

HER2 inhibits the intrinsic apoptotic route, through trophic signalling induced by the serine/threonine kinase Akt, and phosphatidylinositol-3 kinase (PI3K) phosphorylation (Figure 1).[93] Moreover, HER2 blocking agents, used as anticancer drugs, can induce apoptosis by down-regulating factors involved in HER2 signal transduction via Akt and PI3K.[94] Interestingly, the monoclonal antibody trastuzumab, enhances TRAIL-mediated apoptosis by inhibiting Akt, in HER2 overexpressing tumor cell lines.[95]

Hypertrophic growth can serve as a compensatory mechanism for different mechanical, hemodynamic, hormonal and pathologic stimuli. Aortic banding in conditionally mutated mice with a cardiac-restricted HER2 deficiency, did not result in a hypertrophic growth response.[83] This indicates that HER2 is required for myocardial hypertrophy. In analogy in a rat model for LVH induced by aortic banding, HER2, HER4 and neuregulin mRNAs were determined after sacrifice, 6 or 22 weeks after surgical induction of aortic stenosis, and compared to sham-

operated controls.[96] HER2 and HER4 mRNA and protein did not differ at 6 weeks, but at 22 weeks, rats at the edge of developing CHF, had lower HER2 (and HER4) mRNA and protein levels than controls. ANP mRNA increased with the duration of aortic banding, as was to be expected. These results indicate that HER2 is involved in compensatory LVH, prior to CHF.
The precise role of HER2 in human cardiac physiology and disease is still unknown. Myocardial HER2 and HER4 mRNA expression was studied in LV biopsies (RT-PCR) from 36 patients with severe CHF (NYHA class IV) due to ischemic or non-ischemic cardiomyopathy, undergoing left ventricular assist device (LVAD) implantation. HER2 and HER4 were up-regulated after LVAD implantation, whereas HER2 prior to implantation was comparable to healthy controls. HER4 mRNA levels however, were elevated in LVAD candidates.[97] These results are in agreement with data from in vitro and animal studies, suggesting that HER2 is required for normal compensatory mechanisms following diverse pathological stimuli. Recently, in six of 60 severe CHF patients, immunohistochemical expression of HER2 (and HER4) was shown in myocardial biopsies.[98]
A promising new technique for in vivo detection of HER2 is radiolabeled trastuzumab gammacamera imaging. This approach appears to be valuable for prediction of trastuzumab cardiotoxicity in HER2 positive breast cancer patients, when performed before trastuzumab treatment.[99,100] Radiolabeled trastuzumab scintigraphy may also contribute to our understanding of CHF pathophysiology. Furthermore, since trastuzumab enhances anthracycline induced cardiotoxicity, it may be of value for early detection of cardiac injury by anthracyclines. Serum detection of the shed extracellular domain of HER2 is also interesting in this regard and may serve as a prognostic marker for CHF.

HER3 has no active tyrosine kinase binding domain and must rely on heterodimerization for phosphorylation of its tyrosine residues.[101]. Upon neuregulin binding, HER3 and HER2, the preferred heterodimerization partner for HER3, connect and transmit a strong mitogenic signal.[102] Similar to the other HERs, HER3 is involved in normal development of several tissues, including the heart, and is also related to tumor growth. Cardiac defects caused by HER3 deficiency largely resemble HER2-related defects, but are milder.[103] Mice lacking HER3 survive slightly longer (13.5 days) than HER2 deficient mice, but they do show underdeveloped organs.[84] Cardiac endocardial cushion differentiation is incomplete, lacking mesenchyme formation, which results in defective valves. trabecularization is however marginally affected in HER3-deficient mice, and differentiation and thickening of myocardial tissue is only slightly reduced.[84] In the embryonic mouse heart, HER3 is expressed in invading mesenchyme, myocardium and endocardium, but not uniformly in the endocardium. Myocardial cells show weak HER3 expression.[82,87] Explanted developing atrioventricular canal tissue from HER2 and HER3 deficient mice, illustrated that HER2 and HER3 are both

required for normal endocardial cushion formation, since underdeveloped mesenchyme was present in both mutant mouse types. In contrast to HER3-deficient mice, neuregulin-deficient and HER4-deficient mice showed no endocardial cushion defects.[82] In adult and neonatal (1 day postnatal) cardiac tissue, HER3 mRNA has not been detected.[62] In conclusion, HER3 appears to be important in embryonic development of the cardiac valvular system, most likely based on its heterodimerization with HER2.

HER4 has an active tyrosine kinase binding domain.[104] In addition to neuregulins HER4 can also be activated by HB-EGF, betacellulin and epiregulin. HER4 functionality is also required for normal development, growth and differentiation of diverse tissue types, particularly the heart and nervous system. HER4 overexpression is related to tumor growth, e.g. breast and ovarian cancer.[105,106] In murine and human tissues, HER4 is especially present in the heart and brain, and to a lesser extent in epithelial tissues.[105,107] Most studies addressing the role for HER2 in cardiac development, also evaluated HER4 involvement, as their cardiac distribution patterns seem identical. Moreover, mice lacking the HER4 gene,[85] exhibit similar cardiac and neurologic abnormalities as neuregulin deficient and HER2 null mice.[81,87] Homozygous HER4 null mice also do not survive beyond embryonic development and die before day 10.5. In these mice, aborted ventricular trabecularization leading to decreased embryonic blood flow, is the most important cardiac abnormality. Apart from slightly reduced endocardial cushion size, there are no apparent abnormalities of the ventricular walls and endocardium.[85] In rats, HER2 and HER4 mRNA and cDNA is expressed in fetal and adult isolated ventricular myocytes. Notably, HER4 mRNA is expressed consistently higher than HER2, in rat cardiomyocytes.[62] Soluble recombinant human neuregulin induces a hypertrophic growth response and decreases cardiomyocyte apoptosis, in rat ventricular myocytes.[62] In rats with left ventricular hypertrophy as a result of banding-induced aortic stenosis, HER4 and HER2 are down-regulated, early in the development of CHF (22 weeks aortic banding). During compensatory hypertrophy (6 weeks aortic banding), HER2 and HER4 mRNA levels are readily detectable.[96] The evidence described above indicates a role for HER4 in cardiac development and physiology, and stress-adaptation of the heart. heterodimerization may be very important for the ultimate signal to be transduced.

HGFR is the specific tyrosine kinase receptor for HGF, a mesenchymal growth factor that stimulates proliferation and differentiation of several cell types.[108] It was first discovered to hepatocytes. HGFR has features characteristic of the tyrosine kinase growth factor receptor family,[109] and is encoded for by the proto-oncogene c-MET. The HGF-HGFR complex is active in various tissue types and mediates complex biological processes.[110] In the developing heart, it is present at the transcriptional level in pre-myocardial tissue.[111]

Serum HGF levels are elevated within 3 hours after onset of chest pain in patients with acute myocardial infarction. This indicates a role for HGF in myocardial infarction, although increased serum HGF levels have not been observed in other cardiovascular diseases, such as angina pectoris, dilated or hypertrophic cardiomyopathy.[112] Rats subjected to myocardial ischemia (1 hour) and reperfusion show increased HGF mRNA levels in the ischemic reperfused area, with a peak at 3 hours after reperfusion.[113] Plasma HGF levels in these rats are more then 10-fold higher than in controls. Myocardial HGFR mRNA is also increased.
Immunohistochemical HGFR expression in myocardial tissue obtained at autopsy, was higher in 13 patients with a prior myocardial infarction (from 2 hours to 10 years before death), compared to 13 patients who died without heart disease.[114] HGFR overexpression was mainly detected in the periphery of the infarcted area and to a lesser extent in hypertrophic cardiomyocytes. Next to evidence of pro-angiogenic effects of HGF, and beneficial effects of HGF in ventricular remodelling,[115] these results suggest a protective role for HGF in cardiac ischemia. In patients admitted with a CHF exacerbation, serum and peripheral blood mononuclear cells HGF levels are higher than in controls.[116] Furthermore, angiotensin converting enzyme (ACE) inhibitor treatment of CHF patients increases serum HGF levels.[117] Decreased HGF serum levels are associated with increased Ang II in these patients. HGF inhibits apoptosis in non-cardiac cell types,[118] inactivates Bax and causes Bcl-xL up-regulation.[119] In vitro, HGF inhibits cardiomyocyte apoptosis.[120] Adult rat ventricular cardiomyocytes exposed to HGF, exhibit increased Erk phosphorylation and activation of GATA-4, a survival-promoting transcription factor. Simultaneously, Bcl-xL expression is increased.[120] In addition, in anthracycline-treated isolated murine cardiomyocytes, apoptosis is attenuated by adenoviral induction of GATA-4.[121] In accordance with the HERs, HGF appears to be important for cardiomyocyte survival, probably by preventing cardiac apoptosis.

COX-2 (Cyclooxygenase-2) is the rate limiting enzyme in prostaglandin synthesis.[122] Two COX isoforms have been identified, namely COX-1, which is constitutively expressed in myocardial tissue, and the inducible COX-2.[123]. COX-1 plays an important role in protecting the gastroduodenal mucosa, whereas COX-2 is an immediate-early response gene. COX-2 is frequently overexpressed in human tumours,[124] and can be induced rapidly in response to tumor promotors, such as cytokines (e.g. TNFα)[125] and growth factors (Figure 1, 2 and 5).[126] Its cellular expression is regulated by MAPK.[127] Studies in human colon cancer revealed that COX-2 is involved in HER2 cross-talk (Figure 1).[128] In human tumor cell lines, COX-2 is also involved in apoptosis prevention, by increasing Akt.[129] For this reason, COX-2 is a hot topic in anticancer research and is promising in this regard.
To date, COX-2 has particularly been investigated for its role in inflammation, rheumatoid disorders and malignant tumors. In the heart, its role is still controversial. Myocardial COX-2 protein expression is increased in patients with

myocardial ischemia, dilated cardiomyopathy and CHF, especially in areas of inflammation and scarring.[130] Selective COX-2 inhibitor treatment in a rat myocardial infarction model, improved left ventricular end-diastolic and systolic pressure, and was related to reduced infarct size.[131] This suggests that COX-2 has deleterious effects on the heart. In contrast, submission of rat neonatal cardiomyocytes to oxidative stress by hydrogen peroxide or doxorubicin, increases COX-2 mRNA and protein expression, and augments prostacyclin formation. Selective COX-2 inhibition (NS-398) or Erk inhibition (PD098059) completely abolishes prostacyclin formation.[132] COX-2 inhibition worsens doxorubicin-mediated cardiotoxicity in rats, based on a rise in plasma troponin-T, serum lactate dehydrogenase and cardiomyocyte apoptosis.[133] COX-2 increases cardiomyocyte resistance to sublethal ischemic stress (ischemic preconditioning). In a rabbit ischemic preconditioning model, myocardial COX-2 mRNA levels in preconditioned areas increased after one hour of ischemic stress. COX-2 mRNA levels returned to normal after 24 hours. COX-2 protein expression was also increased, 24 hours after ischemia. This was associated with higher COX-2 activity, determined by arachidonic acid metabolite levels. Administration of a selective COX-2 inhibitor (NS-398 or celecoxib) completely abrogated the increased COX-2 activity. In the preconditioned rabbits, COX-2 inhibition impaired recovery of myocardial function, represented by systolic wall thickening.[134]

Whether or not COX-2 is of benefit to the heart remains subject to debate. In patients with cardiac disease, NSAIDs, which non-selectively inhibit COX, increase the risk of CHF.[135] A trial in 8,076 patients, comparing gastrointestinal side-effects of a selective (rofecoxib) with a non-selective COX-2 inhibitor (naproxen) for rheumatoid arthritis, showed an increased relative risk of developing cardiovascular events in the rofecoxib group of 2.37 ($P = 0.0016$).[136] However, the results of 2 trials in patients with rheumatoid arthritis, evaluating celecoxib versus non-selective COX-2 inhibition (ibuprofen or diclofenac), did not support this.[137,138]

The role for COX-2 in cardiac pathophysiology is particularly interesting, all the more based on the apparent pivotal role for COX-2 in the crosstalk between cytokines and growth factor receptors.

CONCLUSION

In anti-cancer treatment, cardiotoxicity may be a side effect of compounds that inhibit growth factor receptor signalling. This may be the case for selective COX-2 inhibitors, but also for recently developed tyrosine kinase inhibitors and HER-blockers. The tyrosine kinase inhibiting HER1 blocker ZD1839 (Iressa®) for instance, is used in patients with lung cancer and other solid tumor types. To date, increased incidence of CHF is not observed. Nevertheless, since long-term follow-

up data are still limited, inherent cardiotoxicity cannot be discarded. Currently, several dual HER1-HER2 inhibitors, and a pan HER-blocker are studied in the clinic. In phase I, toxicity profiles of these agents were mild and cardiotoxicity has not (yet) been reported. However, cardiotoxicity must be anticipated and these agents should be applied with caution in patients, especially when concurrent cardiac disease is present. In addition, it is very likely that targeted anti-tumor drugs will be combined with chemotherapeutic agents, which may further increase the risk of cardiotoxicity.

Molecular mechanisms such as apoptosis deserve to gain increasing interest in cardiac pathophysiology as well. All the more since beneficial effects of stem cell transplantation in the treatment of myocardial infarction, suggest that the myocardium can be regenerated.[139,140] Most of what is known to date regarding apoptotic pathways, stems from embryology and cancer research. Addressing growth factor receptor pathways, including COX-2, is also of particular interest for developing new therapies for cardiac disease. In vitro and animal studies indicate great potential for neuregulin in the CHF treatment in the near future. Adversely, cellular overgrowth may emerge as a major limitation for the use of (systemic) neuregulins.

REFERENCES

1. Kostin S, Pool L, Elsasser A, et al. Myocytes die by multiple mechanisms in failing human hearts. Circ Res 2003; 92: 715-724.
2. Klionsky DJ, Emr SD. Autophagy as a regulated pathway of cellular degradation. Science 2000; 290: 1717-1721.
3. Kerr JF, Wyllie AH, Currie AR. Apoptosis: a basic biological phenomenon with wide-ranging implications in tissue kinetics. Br J Cancer 1972; 26: 239-257.
4. Narula J, Haider N, Virmani R, et al. Apoptosis in myocytes in end-stage heart failure. N Engl J Med 1996; 335: 1182-1189.
5. Olivetti G, Abbi R, Quaini F, et al. Apoptosis in the failing human heart. N Engl J Med 1997; 336: 1131-1141.
6. Garcia-Calvo M, Peterson EP, Leiting B, et al. Inhibition of human caspases by peptide-based and macromolecular inhibitors. J Biol Chem 1998; 273: 32608-32613.
7. Bialik S, Cryns VL, Drincic A, et al. The mitochondrial apoptotic pathway is activated by serum and glucose deprivation in cardiac myocytes. Circ Res 1999; 85: 403-414.
8. Narula J, Pandey P, Arbustini E, et al. Apoptosis in heart failure: release of cytochrome c from mitochondria and activation of caspase-3 in human cardiomyopathy. Proc Natl Acad Sci U S A 1999; 96: 8144-8149.
9. Irmler M, Thome M, Hahne M, et al. Inhibition of death receptor signals by cellular FLIP. Nature 1997; 388: 190-195.
10. Zou H, Henzel WJ, Liu X, Lutschg A, Wang X. Apaf-1, a human protein homologous to C. elegans CED-4, participates in cytochrome c-dependent activation of caspase-3. Cell 1997; 90: 405-413.
11. Adams JM, Cory S. The Bcl-2 protein family: arbiters of cell survival. Science 1998; 281: 1322-1326.
12. Kirshenbaum LA, de Moissac D. The bcl-2 gene product prevents programmed cell death of ventricular myocytes. Circulation 1997; 96: 1580-1585.
13. Nakayama K, Nakayama K, Negishi I, et al. Targeted disruption of Bcl-2 alpha beta in mice: occurrence of gray hair, polycystic kidney disease, and lymphocytopenia. Proc Natl Acad Sci U S A 1994; 91: 3700-3704.
14. Li H, Zhu H, Xu CJ, Yuan J. Cleavage of BID by caspase 8 mediates the mitochondrial damage in the Fas pathway of apoptosis. Cell 1998; 94: 491-501.
15. Reed JC. Dysregulation of apoptosis in cancer. J Clin Oncol 1999; 17: 2941-2953.
16. Long X, Boluyt MO, Hipolito ML, et al. p53 and the hypoxia-induced apoptosis of cultured neonatal rat cardiac myocytes. J Clin Invest 1997; 99: 2635-2643.
17. Ashkenazi A. Targeting death and decoy receptors of the tumour-necrosis factor superfamily. Nat Rev Cancer 2002; 2: 420-430.
18. Owen-Schaub LB, Zhang W, Cusack JC, et al. Wild-type human p53 and a temperature-sensitive mutant induce Fas/APO-1 expression. Mol Cell Biol 1995; 15: 3032-3040.
19. Pulkki KJ. Cytokines and cardiomyocyte death. Ann Med 1997; 29: 339-343.
20. Scheubel RJ, Bartling B, Simm A, et al. Apoptotic pathway activation from mitochondria and death receptors without caspase-3 cleavage in failing human myocardium: fragile balance of myocyte survival? J Am Coll Cardiol 2002; 39: 481-488.
21. Chinnaiyan AM, O'Rourke K, Tewari M, Dixit VM. FADD, a novel death domain-containing protein, interacts with the death domain of Fas and initiates apoptosis. Cell 1995; 81: 505-512.
22. Kapadia S, Lee J, Torre-Amione G, et al. Tumor necrosis factor-alpha gene and protein expression in adult feline myocardium after endotoxin administration. J Clin Invest 1995; 96: 1042-1052.
23. Baxter GT, Kuo RC, Jupp OJ, Vandenabeele P, MacEwan DJ. Tumor necrosis factor-alpha mediates both apoptotic cell death and cell proliferation in a human hematopoietic cell line dependent on mitotic activity and receptor subtype expression. J Biol Chem 1999; 274: 9539-9547.
24. Hirschl MM, Gwechenberger M, Binder T, et al. Assessment of myocardial injury by serum tumour necrosis factor alpha measurements in acute myocardial infarction. Eur Heart J 1996; 17: 1852-1859.
25. Levine B, Kalman J, Mayer L, Fillit HM, Packer M. Elevated circulating levels of tumor necrosis factor in severe chronic heart failure. N Engl J Med 1990; 323: 236-241.
26. Torre-Amione G, Kapadia S, Lee J, et al. Tumor necrosis factor-alpha and tumor necrosis factor receptors in the failing human heart. Circulation 1996; 93: 704-711.
27. Petretta M, Condorelli GL, Spinelli L, et al. Circulating levels of cytokines and their site of production in patients with mild to severe chronic heart failure. Am Heart J 2000; 140: E28

28. Sliwa K, Skudicky D, Candy G, Wisenbaugh T, Sareli P. Randomised investigation of effects of pentoxifylline on left- ventricular performance in idiopathic dilated cardiomyopathy. Lancet 1998; 351: 1091-1093.
29. Sliwa K, Woodiwiss A, Candy G, et al. Effects of pentoxifylline on cytokine profiles and left ventricular performance in patients with decompensated congestive heart failure secondary to idiopathic dilated cardiomyopathy. Am J Cardiol 2002; 90: 1118-1122.
30. Bozkurt B, Torre-Amione G, Warren MS, et al. Results of targeted anti-tumor necrosis factor therapy with etanercept (ENBREL) in patients with advanced heart failure. Circulation 2001; 103: 1044-1047.
31. Deswal A, Bozkurt B, Seta Y, et al. Safety and efficacy of a soluble P75 tumor necrosis factor receptor (Enbrel, etanercept) in patients with advanced heart failure. Circulation 1999; 99: 3224-3226.
32. Fichtlscherer S, Rossig L, Breuer S, et al. Tumor necrosis factor antagonism with etanercept improves systemic endothelial vasoreactivity in patients with advanced heart failure. Circulation 2001; 104: 3023-3025.
33. Anker SD, Coats AJ. How to RECOVER from RENAISSANCE? The significance of the results of RECOVER, RENAISSANCE, RENEWAL and ATTACH. Int J Cardiol 2002; 86: 123-130.
34. Itoh N, Yonehara S, Ishii A, et al. The polypeptide encoded by the cDNA for human cell surface antigen Fas can mediate apoptosis. Cell 1991; 66: 233-243.
35. Cheng J, Zhou T, Liu C, et al. Protection from Fas-mediated apoptosis by a soluble form of the Fas molecule. Science 1994; 263: 1759-1762.
36. Ogasawara J, Watanabe-Fukunaga R, Adachi M, et al. Lethal effect of the anti-Fas antibody in mice. Nature 1993; 364: 806-809.
37. Rensing-Ehl A, Frei K, Flury R, et al. Local Fas/APO-1 (CD95) ligand-mediated tumor cell killing in vivo. Eur J Immunol 1995; 25: 2253-2258.
38. Wollert KC, Heineke J, Westermann J, et al. The cardiac Fas (APO-1/CD95) Receptor/Fas ligand system : relation to diastolic wall stress in volume-overload hypertrophy in vivo and activation of the transcription factor AP-1 in cardiac myocytes. Circulation 2000; 101: 1172-1178.
39. Yue TL, Ma XL, Wang X, et al. Possible involvement of stress-activated protein kinase signaling pathway and Fas receptor expression in prevention of ischemia/reperfusion-induced cardiomyocyte apoptosis by carvedilol. Circ Res 1998; 82: 166-174.
40. Lee P, Sata M, Lefer DJ, et al. Fas pathway is a critical mediator of cardiac myocyte death and MI during ischemia-reperfusion in vivo. Am J Physiol Heart Circ Physiol 2003; 284: H456-H463
41. Yaniv G, Shilkrut M, Lotan R, et al. Hypoxia predisposes neonatal rat ventricular myocytes to apoptosis induced by activation of the Fas (CD95/Apo-1) receptor: Fas activation and apoptosis in hypoxic myocytes. Cardiovasc Res 2002; 54: 611-623.
42. Filippatos G, Leche C, Sunga R, et al. Expression of FAS adjacent to fibrotic foci in the failing human heart is not associated with increased apoptosis. Am J Physiol 1999; 277: H445-H451
43. Nishigaki K, Minatoguchi S, Seishima M, et al. Plasma Fas ligand, an inducer of apoptosis, and plasma soluble Fas, an inhibitor of apoptosis, in patients with chronic congestive heart failure. J Am Coll Cardiol 1997; 29: 1214-1220.
44. Okuyama M, Yamaguchi S, Nozaki N, et al. Serum levels of soluble form of Fas molecule in patients with congestive heart failure. Am J Cardiol 1997; 79: 1698-1701.
45. Tsutamoto T, Wada A, Maeda K, et al. Relationship between plasma levels of cardiac natriuretic peptides and soluble Fas: plasma soluble Fas as a prognostic predictor in patients with congestive heart failure. J Card Fail 2001; 7: 322-328.
46. Adamopoulos S, Parissis JT, Georgiadis M, et al. Growth hormone administration reduces circulating proinflammatory cytokines and soluble Fas/soluble Fas ligand system in patients with chronic heart failure secondary to idiopathic dilated cardiomyopathy. Am Heart J 2002; 144: 359-364.
47. Badorff C, Ruetten H, Mueller S, et al. Fas receptor signaling inhibits glycogen synthase kinase 3 beta and induces cardiac hypertrophy following pressure overload. J Clin Invest 2002; 109: 373-381.
48. Pitti RM, Marsters SA, Ruppert S, et al. Induction of apoptosis by Apo-2 ligand, a new member of the tumor necrosis factor cytokine family. J Biol Chem 1996; 271: 12687-12690.
49. Pan G, O'Rourke K, Chinnaiyan AM, et al. The receptor for the cytotoxic ligand TRAIL. Science 1997; 276: 111-113.
50. Schneider P, Bodmer JL, Thome M, et al. Characterization of two receptors for TRAIL. FEBS Lett 1997; 416: 329-334.
51. MacFarlane M, Ahmad M, Srinivasula SM, et al. Identification and molecular cloning of two novel receptors for the cytotoxic ligand TRAIL. J Biol Chem 1997; 272: 25417-25420.

52. Degli-Esposti MA, Dougall WC, Smolak PJ, et al. The novel receptor TRAIL-R4 induces NF-kappaB and protects against TRAIL-mediated apoptosis, yet retains an incomplete death domain. Immunity 1997; 7: 813-820.
53. Spierings DC, de Vries EG, Vellenga E, et al. Tissue Distribution of TRAIL and TRAIL Receptors in Normal Chimpanzee and Human Tissues. to be submitted 2003.
54. Jeremias I, Kupatt C, Martin-Villalba A, et al. Involvement of CD95/Apo1/Fas in cell death after myocardial ischemia. Circulation 2000; 102: 915-920.
55. Yndestad A, Kristian DJ, Geir EH, et al. Increased gene expression of tumor necrosis factor superfamily ligands in peripheral blood mononuclear cells during chronic heart failure. Cardiovasc Res 2002; 54: 175-182.
56. Secchiero P, Gonelli A, Carnevale E, et al. TRAIL promotes the survival and proliferation of primary human vascular endothelial cells by activating the Akt and ERK pathways. Circulation 2003; 107: 2250-2256.
57. Zauli G, Pandolfi A, Gonelli A, et al. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) sequentially upregulates nitric oxide and prostanoid production in primary human endothelial cells. Circ Res 2003; 92: 732-740.
58. Zhang XD, Nguyen T, Thomas WD, Sanders JE, Hersey P. Mechanisms of resistance of normal cells to TRAIL induced apoptosis vary between different cell types. FEBS Lett 2000; 482: 193-199.
59. Yarden Y, Sliwkowski MX. Untangling the ErbB signalling network. Nat Rev Mol Cell Biol 2001; 2: 127-137.
60. Tzahar E, Waterman H, Chen X, et al. A hierarchical network of interreceptor interactions determines signal transduction by Neu differentiation factor/neuregulin and epidermal growth factor. Mol Cell Biol 1996; 16: 5276-5287.
61. Olayioye MA, Beuvink I, Horsch K, Daly JM, Hynes NE. ErbB receptor-induced activation of STAT transcription factors is mediated by Src tyrosine kinases. J Biol Chem 1999; 274: 17209-17218.
62. Zhao YY, Sawyer DR, Baliga RR, et al. Neuregulins promote survival and growth of cardiac myocytes. Persistence of ErbB2 and ErbB4 expression in neonatal and adult ventricular myocytes. J Biol Chem 1998; 273: 10261-10269.
63. Russell KS, Stern DF, Polverini PJ, Bender JR. Neuregulin activation of ErbB receptors in vascular endothelium leads to angiogenesis. Am J Physiol 1999; 277: H2205-H2211
64. Miettinen PJ, Berger JE, Meneses J, et al. Epithelial immaturity and multiorgan failure in mice lacking epidermal growth factor receptor. Nature 1995; 376: 337-341.
65. Lau CL. Behavior of embryonic chick heart cells in culture. 3. Spatial distribution of epidermal growth factor in heart muscle cells. Tissue Cell 1994; 26: 203-208.
66. Chen B, Bronson RT, Klaman LD, et al. Mice mutant for Egfr and Shp2 have defective cardiac semilunar valvulogenesis. Nat Genet 2000; 24: 296-299.
67. Goldman B, Mach A, Wurzel J. Epidermal growth factor promotes a cardiomyoblastic phenotype in human fetal cardiac myocytes. Exp Cell Res 1996; 228: 237-245.
68. Thomas WG, Brandenburger Y, Autelitano DJ, et al. Adenoviral-directed expression of the type 1A angiotensin receptor promotes cardiomyocyte hypertrophy via transactivation of the epidermal growth factor receptor. Circ Res 2002; 90: 135-142.
69. Kim J, Eckhart AD, Eguchi S, Koch WJ. Beta-adrenergic receptor-mediated DNA synthesis in cardiac fibroblasts is dependent on transactivation of the epidermal growth factor receptor and subsequent activation of extracellular signal-regulated kinases. J Biol Chem 2002; 277: 32116-32123.
70. Fujino T, Hasebe N, Fujita M, et al. Enhanced expression of heparin-binding EGF-like growth factor and its receptor in hypertrophied left ventricle of spontaneously hypertensive rats. Cardiovasc Res 1998; 38: 365-374.
71. Tanaka N, Masamura K, Yoshida M, et al. A role of heparin-binding epidermal growth factor-like growth factor in cardiac remodeling after myocardial infarction. Biochem Biophys Res Commun 2002; 297: 375-381.
72. Pareja M, Sanchez O, Lorita J, Soley M, Ramirez I. Activated epidermal growth factor receptor (ErbB1) protects the heart against stress-induced injury in mice. Am J Physiol Regul Integr Comp Physiol 2003; 285: R455-R462
73. Dong Z, Brennan A, Liu N, et al. Neu differentiation factor is a neuron-glia signal and regulates survival, proliferation, and maturation of rat Schwann cell precursors. Neuron 1995; 15: 585-596.
74. Hynes NE, Stern DF. The biology of erbB-2/neu/HER-2 and its role in cancer. Biochim Biophys Acta 1994; 1198: 165-184.
75. Slamon DJ, Clark GM, Wong SG, et al. Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235: 177-182.

76. Carter P, Presta L, Gorman CM, et al. Humanization of an anti-p185HER2 antibody for human cancer therapy. Proc Natl Acad Sci U S A 1992; 89: 4285-4289.
77. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344: 783-792.
78. Schneider JW, Chang AY, Rocco TP. Cardiotoxicity in signal transduction therapeutics: erbB2 antibodies and the heart. Semin Oncol 2001; 28: 18-26.
79. Sawyer DB, Zuppinger C, Miller TA, Eppenberger HM, Suter TM. Modulation of anthracycline-induced myofibrillar disarray in rat ventricular myocytes by neuregulin-1beta and anti-erbB2: potential mechanism for trastuzumab-induced cardiotoxicity. Circulation 2002; 105: 1551-1554.
80. Wu JY, Yu H, Cohen IS. Epidermal growth factor increases i(f) in rabbit SA node cells by activating a tyrosine kinase. Biochim Biophys Acta 2000; 1463: 15-19.
81. Lee KF, Simon H, Chen H, et al. Requirement for neuregulin receptor erbB2 in neural and cardiac development. Nature 1995; 378: 394-398.
82. Camenisch TD, Schroeder JA, Bradley J, Klewer SE, McDonald JA. Heart-valve mesenchyme formation is dependent on hyaluronan-augmented activation of ErbB2-ErbB3 receptors. Nat Med 2002; 8: 850-855.
83. Ozcelik C, Erdmann B, Pilz B, et al. Conditional mutation of the ErbB2 (HER2) receptor in cardiomyocytes leads to dilated cardiomyopathy. Proc Natl Acad Sci U S A 2002; 99: 8880-8885.
84. Erickson SL, O'Shea KS, Ghaboosi N, et al. ErbB3 is required for normal cerebellar and cardiac development: a comparison with ErbB2-and heregulin-deficient mice. Development 1997; 124: 4999-5011.
85. Gassmann M, Casagranda F, Orioli D, et al. Aberrant neural and cardiac development in mice lacking the ErbB4 neuregulin receptor. Nature 1995; 378: 390-394.
86. Kramer R, Bucay N, Kane DJ, et al. Neuregulins with an Ig-like domain are essential for mouse myocardial and neuronal development. Proc Natl Acad Sci U S A 1996; 93: 4833-4838.
87. Meyer D, Birchmeier C. Multiple essential functions of neuregulin in development. Nature 1995; 378: 386-390.
88. Chan R, Hardy WR, Laing MA, Hardy SE, Muller WJ. The catalytic activity of the ErbB-2 receptor tyrosine kinase is essential for embryonic development. Mol Cell Biol 2002; 22: 1073-1078.
89. Morris JK, Lin W, Hauser C, et al. Rescue of the cardiac defect in ErbB2 mutant mice reveals essential roles of ErbB2 in peripheral nervous system development. Neuron 1999; 23: 273-283.
90. Woldeyesus MT, Britsch S, Riethmacher D, et al. Peripheral nervous system defects in erbB2 mutants following genetic rescue of heart development. Genes Dev 1999; 13: 2538-2548.
91. Wu CF, Bishopric NH, Pratt RE. Atrial natriuretic peptide induces apoptosis in neonatal rat cardiac myocytes. J Biol Chem 1997; 272: 14860-14866.
92. Crone SA, Zhao YY, Fan L, et al. ErbB2 is essential in the prevention of dilated cardiomyopathy. Nat Med 2002; 8: 459-465.
93. Bacus SS, Altomare DA, Lyass L, et al. AKT2 is frequently upregulated in HER-2/neu-positive breast cancers and may contribute to tumor aggressiveness by enhancing cell survival. Oncogene 2002; 21: 3532-3540.
94. Yakes FM, Chinratanalab W, Ritter CA, et al. Herceptin-induced inhibition of phosphatidylinositol-3 kinase and Akt Is required for antibody-mediated effects on p27, cyclin D1, and antitumor action. Cancer Res 2002; 62: 4132-4141.
95. Cuello M, Ettenberg SA, Clark AS, et al. Down-regulation of the erbB-2 receptor by trastuzumab (herceptin) enhances tumor necrosis factor-related apoptosis-inducing ligand- mediated apoptosis in breast and ovarian cancer cell lines that overexpress erbB-2. Cancer Res 2001; 61: 4892-4900.
96. Rohrbach S, Yan X, Weinberg EO, et al. Neuregulin in cardiac hypertrophy in rats with aortic stenosis. Differential expression of erbB2 and erbB4 receptors. Circulation 1999; 100: 407-412.
97. Uray IP, Connelly JH, Thomazy V, et al. Left ventricular unloading alters receptor tyrosine kinase expression in the failing human heart. J Heart Lung Transplant 2002; 21: 771-782.
98. Fuchs IB, Landt S, Bueler H, et al. Analysis of HER2 and HER4 in human myocardium to clarify the cardiotoxicity of trastuzumab (Herceptin). Breast Cancer Res Treat 2003; 82: 23-28.
99. Behr TM, Behe M, Wormann B. Trastuzumab and breast cancer. N Engl J Med 2001; 345: 995-996.
100. Lub-De Hooge MN, Perik PJ, Gietema JA, et al. Biodistribution and pharmacokinetics of radiolabeled trastuzumab in HER2-positive metastatic breast cancer. Proc Am Soc Clin Oncol 2004;22:abstr 3063.
101. Wallasch C, Weiss FU, Niederfellner G, et al. Heregulin-dependent regulation of HER2/neu oncogenic signaling by heterodimerization with HER3. EMBO J 1995; 14: 4267-4275.
102. Karunagaran D, Tzahar E, Beerli RR, et al. ErbB-2 is a common auxiliary subunit of NDF and EGF receptors: implications for breast cancer. EMBO J 1996; 15: 254-264.

103. Britsch S, Li L, Kirchhoff S, et al. The ErbB2 and ErbB3 receptors and their ligand, neuregulin-1, are essential for development of the sympathetic nervous system. Genes Dev 1998; 12: 1825-1836.
104. Burden S, Yarden Y. Neuregulins and their receptors: a versatile signaling module in organogenesis and oncogenesis. Neuron 1997; 18: 847-855.
105. Plowman GD, Culouscou JM, Whitney GS, et al. Ligand-specific activation of HER4/p180erbB4, a fourth member of the epidermal growth factor receptor family. Proc Natl Acad Sci U S A 1993; 90: 1746-1750.
106. Gilmour LM, Macleod KG, McCaig A, et al. Neuregulin expression, function, and signaling in human ovarian cancer cells. Clin Cancer Res 2002; 8: 3933-3942.
107. Pinkas-Kramarski R, Eilam R, Alroy I, et al. Differential expression of NDF/neuregulin receptors ErbB-3 and ErbB-4 and involvement in inhibition of neuronal differentiation. Oncogene 1997; 15: 2803-2815.
108. Nakamura Y, Morishita R, Higaki J, et al. Hepatocyte growth factor is a novel member of the endothelium-specific growth factors: additive stimulatory effect of hepatocyte growth factor with basic fibroblast growth factor but not with vascular endothelial growth factor. J Hypertens 1996; 14: 1067-1072.
109. Park M, Dean M, Kaul K, et al. Sequence of MET protooncogene cDNA has features characteristic of the tyrosine kinase family of growth-factor receptors. Proc Natl Acad Sci U S A 1987; 84: 6379-6383.
110. Matsumoto K, Nakamura T. Emerging multipotent aspects of hepatocyte growth factor. J Biochem (Tokyo) 1996; 119: 591-600.
111. Rappolee DA, Iyer A, Patel Y. Hepatocyte growth factor and its receptor are expressed in cardiac myocytes during early cardiogenesis. Circ Res 1996; 78: 1028-1036.
112. Matsumori A, Furukawa Y, Hashimoto T, et al. Increased circulating hepatocyte growth factor in the early stage of acute myocardial infarction. Biochem Biophys Res Commun 1996; 221: 391-395.
113. Ono K, Matsumori A, Shioi T, Furukawa Y, Sasayama S. Enhanced expression of hepatocyte growth factor/c-Met by myocardial ischemia and reperfusion in a rat model. Circulation 1997; 95: 2552-2558.
114. Sato T, Tani Y, Murao S, et al. Focal enhancement of expression of c-Met/hepatocyte growth factor receptor in the myocardium in human myocardial infarction. Cardiovasc Pathol 2001; 10: 235-240.
115. Yasuda S, Goto Y, Baba T, et al. Enhanced secretion of cardiac hepatocyte growth factor from an infarct region is associated with less severe ventricular enlargement and improved cardiac function. J Am Coll Cardiol 2000; 36: 115-121.
116. Ueno S, Ikeda U, Hojo Y, et al. Serum hepatocyte growth factor levels are increased in patients with congestive heart failure. J Card Fail 2001; 7: 329-334.
117. Yasuda S, Goto Y, Sumida H, et al. Angiotensin-converting enzyme inhibition restores hepatocyte growth factor production in patients with congestive heart failure. Hypertension 1999; 33: 1374-1378.
118. Tanaka T, Mizuno K, Umesaki N, Ogita S. Suppressed apoptotic susceptibility in human endometrial epithelial cells pretreated with hepatocyte growth factor. Clin Exp Obstet Gynecol 1998; 25: 125-128.
119. Liu Y. Hepatocyte growth factor promotes renal epithelial cell survival by dual mechanisms. Am J Physiol 1999; 277: F624-F633
120. Kitta K, Day RM, Kim Y, et al. Hepatocyte growth factor induces GATA-4 phosphorylation and cell survival in cardiac muscle cells. J Biol Chem 2003; 278: 4705-4712.
121. Kim Y, Ma AG, Kitta K, et al. Anthracycline-induced suppression of GATA-4 transcription factor: implication in the regulation of cardiac myocyte apoptosis. Mol Pharmacol 2003; 63: 368-377.
122. Smith WL, Garavito RM, DeWitt DL. Prostaglandin endoperoxide H synthases (cyclooxygenases)-1 and -2. J Biol Chem 1996; 271: 33157-33160.
123. Raz A, Wyche A, Siegel N, Needleman P. Regulation of fibroblast cyclooxygenase synthesis by interleukin-1. J Biol Chem 1988; 263: 3022-3028.
124. Koki AT, Masferrer JL. Celecoxib: a specific COX-2 inhibitor with anticancer properties. Cancer Control 2002; 9: 28-35.
125. Yamamoto K, Arakawa T, Ueda N, Yamamoto S. Transcriptional roles of nuclear factor kappa B and nuclear factor- interleukin-6 in the tumor necrosis factor alpha-dependent induction of cyclooxygenase-2 in MC3T3-E1 cells. J Biol Chem 1995; 270: 31315-31320.
126. Di Popolo A, Memoli A, Apicella A, et al. IGF-II/IGF-I receptor pathway up-regulates COX-2 mRNA expression and PGE2 synthesis in Caco-2 human colon carcinoma cells. Oncogene 2000; 19: 5517-5524.

127. Degousee N, Martindale J, Stefanski E, et al. MAP kinase 6-p38 MAP kinase signaling cascade regulates cyclooxygenase-2 expression in cardiac myocytes in vitro and in vivo. Circ Res 2003; 92: 757-764.
128. Vadlamudi R, Mandal M, Adam L, et al. Regulation of cyclooxygenase-2 pathway by HER2 receptor. Oncogene 1999; 18: 305-314.
129. Ricchi P, Zarrilli R, Di Palma A, Acquaviva AM. Nonsteroidal anti-inflammatory drugs in colorectal cancer: from prevention to therapy. Br J Cancer 2003; 88: 803-807.
130. Wong SC, Fukuchi M, Melnyk P, Rodger I, Giaid A. Induction of cyclooxygenase-2 and activation of nuclear factor-kappaB in myocardium of patients with congestive heart failure. Circulation 1998; 98: 100-103.
131. Saito T, Rodger IW, Shennib H, et al. Cyclooxygenase-2 (COX-2) in acute myocardial infarction: cellular expression and use of selective COX-2 inhibitor. Can J Physiol Pharmacol 2003; 81: 114-119.
132. Adderley SR, Fitzgerald DJ. Oxidative damage of cardiomyocytes is limited by extracellular regulated kinases 1/2-mediated induction of cyclooxygenase-2. J Biol Chem 1999; 274: 5038-5046.
133. Dowd NP, Scully M, Adderley SR, Cunningham AJ, Fitzgerald DJ. Inhibition of cyclooxygenase-2 aggravates doxorubicin-mediated cardiac injury in vivo. J Clin Invest 2001; 108: 585-590.
134. Shinmura K, Tang XL, Wang Y, et al. Cyclooxygenase-2 mediates the cardioprotective effects of the late phase of ischemic preconditioning in conscious rabbits. Proc Natl Acad Sci U S A 2000; 97: 10197-10202.
135. Bleumink GS, Feenstra J, Sturkenboom MC, Stricker BH. Nonsteroidal anti-inflammatory drugs and heart failure. Drugs 2003; 63: 525-534.
136. Bombardier C, Laine L, Reicin A, et al. Comparison of upper gastrointestinal toxicity of rofecoxib and naproxen in patients with rheumatoid arthritis. VIGOR Study Group. N Engl J Med 2000; 343: 1520-8, 2.
137. Silverstein FE, Faich G, Goldstein JL, et al. Gastrointestinal toxicity with celecoxib vs nonsteroidal anti- inflammatory drugs for osteoarthritis and rheumatoid arthritis: the CLASS study: A randomized controlled trial. Celecoxib Long-term Arthritis Safety Study. JAMA 2000; 284: 1247-1255.
138. White WB, Faich G, Whelton A, et al. Comparison of thromboembolic events in patients treated with celecoxib, a cyclooxygenase-2 specific inhibitor, versus ibuprofen or diclofenac. Am J Cardiol 2002; 89: 425-430.
139. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. Circulation 2002; 106: 1913-1918.
140. Assmus B, Schachinger V, Teupe C, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction (TOPCARE-AMI). Circulation 2002; 106: 3009-3017.