Cardiac hormones for the treatment of cancer

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Abstract

Four cardiac hormones, namely atrial natriuretic peptide, vessel dilator, kaliuretic peptide, and long-acting natriuretic peptide, reduce up to 97% of all cancer cells in vitro. These four cardiac hormones eliminate up to 86% of human small-cell lung carcinomas, two-thirds of human breast cancers, and up to 80% of human pancreatic adenocarcinomas growing in athymic mice. Their anticancer mechanisms of action, after binding to specific receptors on cancer cells, include targeting the rat sarcoma-bound GTP (RAS) (95% inhibition)–mitogen-activated protein kinase kinase 1/2 (MEK 1/2) (98% inhibition)–extracellular signal-related kinase 1/2 (ERK 1/2) (96% inhibition) cascade in cancer cells. They also inhibit MAPK9, i.e. c-Jun N-terminal kinase 2. They are dual inhibitors of vascular endothelial growth factor (VEGF) and its VEGFR2 receptor (up to 89%). One of the downstream targets of VEGF is β-catenin, which they reduce up to 88%. The WNT pathway is inhibited up to 68% and secreted frizzled-related protein 3 decreased up to 84% by the four cardiac hormones. AKT, a serine/threonine protein kinase, is reduced up to 64% by the cardiac hormones. STAT3, a final ‘switch’ that activates gene expression that leads to malignancy, is decreased by up to 88% by the cardiac hormones. STAT3 is specifically decreased as they do not affect STAT1. There is a cross-talk between the RAS–MEK 1/2–ERK 1/2 kinase cascade, VEGF, β-catenin, WNT, JNK, and STAT pathways and each of these pathways is inhibited by the cardiac hormones.

Introduction

The heart is a sophisticated endocrine gland that synthesizes at least six peptide hormones by three different genes (Brenner et al. 1990, Gardner et al. 1997, Vesely 2002). The cardiac hormones synthesized by these three genes are stored as three different prohormones, namely the 126 amino acid (a.a.) atrial natriuretic peptide (ANP), 108 a.a. brain natriuretic peptide (BNP), and 103 a.a. C-type natriuretic peptide (CNP) prohormones (Brenner et al. 1990, Gardner et al. 1997, Vesely 2002).

Within the 126 a.a. ANP prohormone are four peptide hormones (Fig. 1). The main physiological properties of these four peptide hormones after their release from the heart are blood pressure regulation and the maintenance of plasma volume in animals (Vesely et al. 1987, Martin et al. 1990, Gunning et al. 1992, Benjamin & Peterson 1995, Zeidel 1995, Villarreal et al. 1999, Dietz et al. 2001) and humans (Vesely et al. 1994a,b, 1998). The cardiac hormones, synthesized in the atrium of the heart, are...
The atrial natriuretic peptide (ANP) gene in the heart codes for a 126 amino acid (a.a.) prohormone which through proteolytic processing results in the formation of the four cardiac hormones. These four cardiac hormones are: long-acting natriuretic peptide (LANP), consisting of the first 30 a.a. from the N-terminus of the 126 a.a. prohormone; vessel dilator (VDL), consisting of a.a. 31–67 of the prohormone; kaliuretic peptide (KP), consisting of a.a. 79–98 of this prohormone; and ANP, consisting of a.a. 99–126 of the 126 a.a. prohormone. Reprinted with permission from Sun Y, Eichelbaum EJ, Wang H & Vesely DL 2006. Anticancer Research 26 3217–3222.

Figure 1

The atrial natriuretic peptide (ANP) gene in the heart codes for a 126 amino acid (a.a.) prohormone which through proteolytic processing results in the formation of the four cardiac hormones. These four cardiac hormones are: long-acting natriuretic peptide (LANP), consisting of the first 30 a.a. from the N-terminus of the 126 a.a. prohormone; vessel dilator, a.a. 31–67; kaliuretic peptide, a.a. 79–98 of the prohormone; vessel dilator, a.a. 31–67; kaliuretic peptide, a.a. 79–98; and ANP, a.a. 99–126 of the 126 a.a. prohormone. Reprinted with permission from Sun Y, Eichelbaum EJ, Wang H & Vesely DL 2006. Vessel dilator and kaliuretic peptide inhibit activation of ERK 1/2 in human prostate cancer cells. Anticancer Research 26 3217–3222.

Figure 2

Structure of natriuretic peptide receptor-A (NPR-A, active receptor). The extracellular portion of the receptor (441 amino acids) binds atrial natriuretic peptide (ANP) from the circulation, which activates the catalytic portion of guanylate cyclase within the receptor itself on the inside of the cell membrane and then catalyzes the conversion of GTP to cyclic GMP. The structure illustrated was drawn utilizing the amino acid sequences determined for the NPR-A receptor. Reprinted with permission of Vesely DL 1992. Guanylate cyclase converts GTP to cyclic GMP (Waldman et al. 1984). Cyclic GMP has a strong anticancer effect of decreasing human pancreatic cancer cell volume in vivo by 95% (Vesely et al. 2004). Cyclic GMP inhibits the activation of kinases in the Ras–MEK 1/2–ERK 1/2 kinase cascade that mediate the growth of cancers (Fig. 3). Thus, cyclic GMP blocks the conversion of inactive Ras-GDP to active Ras-GTP by 89% (Sun et al. 2009b), inhibits the phosphorylation of MEK 1/2 kinases by 93% (Sun et al. 2007b), and inhibits 83% of the phosphorylation of ERK 1/2 kinases (Sun et al. 2006b).

Because guanylate cyclase is part of the NPR-A receptor itself (Fig. 2), one would expect that the stimulation of the NPR-A receptor would inhibit the proliferation and growth of cancer cells via increasing cyclic GMP. With respect to this, knockout of the NPR-A receptor results in no growth of implanted cancer cells in mice (Wang et al. 2011). The NPR-C receptor, on the other hand, does not contain guanylate cyclase as the NPR-A (NPR-A; Fig. 2; Vesely 1992). Guanylate cyclase converts GTP to cyclic GMP (Waldman et al. 1984). Cyclic GMP has a strong anticancer effect of decreasing human pancreatic cancer cell volume in vivo by 95% (Vesely et al. 2004). Cyclic GMP inhibits the activation of kinases in the Ras–MEK 1/2–ERK 1/2 kinase cascade that mediate the growth of cancers (Fig. 3). Thus, cyclic GMP blocks the conversion of inactive Ras-GDP to active Ras-GTP by 89% (Sun et al. 2009b), inhibits the phosphorylation of MEK 1/2 kinases by 93% (Sun et al. 2007b), and inhibits 83% of the phosphorylation of ERK 1/2 kinases (Sun et al. 2006b).

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The NPR-C receptor, however, has been reported to have in vitro antiproliferative effects (Gower et al. 2006). Although the mechanism(s) of how it might be involved has not been determined, it has been shown that the NPR-C receptor is downregulated by prostaglandin E2 (Gower et al. 2006). With respect to the possible mechanism(s) of how the NPR-C receptor might have antiproliferative effects, it is known that not all of the biological effects of the cardiac hormones are mediated by cyclic GMP but some are mediated via prostaglandin E2 (Gunning et al. 1992, Chiou & Vesely 1995). Although it does appear that all of the anticancer effects of the cardiac hormones are mediated by cyclic GMP, the abilities of vessel dilator and kaliuretic peptide to cause a natriuresis are not mediated by cyclic GMP but rather via enhancing prostaglandin E2, which is synthesized secondary to vessel dilator and kaliuretic peptide (Gunning et al. 1992, Chiou & Vesely 1995). The ability of prostaglandin E2 to downregulate the NPR-C receptor, i.e. the clearance receptor for clearing ANP (Gower et al. 2006), should result in less clearance of ANP, thus allowing more circulating ANP to stimulate the NPR-A receptor as a possible mechanism of how the NPR-C receptor may help decrease cell proliferation.

Cardiac hormones inhibit RAS-GTP, MEK 1/2, and ERK 1/2 kinases of the RAS–MEK 1/2–ERK 1/2 kinase cascade by 95–98%. These multiple kinase inhibitors are also strong inhibitors (i.e. 91%) of DNA synthesis within cancer cells. Other targets which the cardiac hormones inhibit within cancer cells are VEGF, the VEGFR2 receptor, β-catenin, secreted frizzled receptor, JNK, STAT3, and the WNT pathway. RTK, tyrosine kinase receptor; SRC, rous sarcoma viral proto-oncogene tyrosine kinase; SHC, rous sarcoma SH2 C-terminal binding domain adapter protein; GRB2, growth factor receptor-bound protein 2; SOS, son of sevenless gene; RAS-GDP, rat sarcoma-bound GDP; RAS-GTP, rat sarcoma-bound GTP; RAF, rapidly accelerated fibrosarcoma serine/threonine protein kinase; MOS, Moloney murine sarcoma virus serine/threonine protein kinase; MEK 1/2, mitogen-activated protein kinase kinase 1/2; ERK 1/2, extracellular signal-related kinases 1/2; VEGF, vascular endothelial growth factor; sFRP-3, secreted frizzled-related protein-3; JNK, c-Jun N-terminal kinases; AKT, AK mouse strain with ‘t’ for thymoma. Modified with permission from Sun Y, Eichelbaum EJ, Lenz A, Wang H & Vesely DL 2010 Epidermal growth factor’s activation of RAS is inhibited by four cardiac hormones. European Journal of Clinical Investigation 40 408–413.

Figure 3
Cardiac hormones inhibit RAS-GTP, MEK 1/2, and ERK 1/2 kinases of the RAS–MEK 1/2–ERK 1/2 kinase cascade by 95–98%. These multiple kinase inhibitors are also strong inhibitors (i.e. 91%) of DNA synthesis within cancer cells. Other targets which the cardiac hormones inhibit within cancer cells are VEGF, the VEGFR2 receptor, β-catenin, secreted frizzled receptor, JNK, STAT3, and the WNT pathway. RTK, tyrosine kinase receptor; SRC, rous sarcoma viral proto-oncogene tyrosine kinase; SHC, rous sarcoma SH2 C-terminal binding domain adapter protein; GRB2, growth factor receptor-bound protein 2; SOS, son of sevenless gene; RAS-GDP, rat sarcoma-bound GDP; RAS-GTP, rat sarcoma-bound GTP; RAF, rapidly accelerated fibrosarcoma serine/threonine protein kinase; MOS, Moloney murine sarcoma virus serine/threonine protein kinase; MEK 1/2, mitogen-activated protein kinase kinase 1/2; ERK 1/2, extracellular signal-related kinases 1/2; VEGF, vascular endothelial growth factor; sFRP-3, secreted frizzled-related protein-3; JNK, c-Jun N-terminal kinases; AKT, AK mouse strain with ‘t’ for thymoma. Modified with permission from Sun Y, Eichelbaum EJ, Lenz A, Wang H & Vesely DL 2010 Epidermal growth factor’s activation of RAS is inhibited by four cardiac hormones. European Journal of Clinical Investigation 40 408–413.
Anticancer effects of the cardiac hormones in vitro

LANP, vessel dilator, kaliuretic peptide, and ANP decrease the number, i.e., eliminate, up to 97% of pancreatic (Vesely et al. 2003), breast (Vesely et al. 2005b), colon (Gower et al. 2005), kidney (Vesely et al. 2006c), prostate (Vesely et al. 2005a), ovarian (Vesely et al. 2007b,d), small-cell lung (Vesely et al. 2005b), and squamous cell lung carcinoma cells in vitro (Vesely et al. 2006b). In addition, they decrease glioblastomas of the brain (Vesely et al. 2007a,c), medullary thyroid carcinomas (Eichelbaum et al. 2006), and angiosarcoma of the heart (Vesely et al. 2006a). There is no proliferation of the cancer cells that are not eliminated when examined for 3 days following the elimination of other cancer cells secondary to the cardiac hormones (Vesely et al. 2005a). ANP also decreases hepatoblastoma cells in culture (Rashed et al. 1993); however, the other cardiac hormones have not been investigated for their effects upon hepatoblastoma cells in culture. Serafino et al. (2012) have confirmed that ANP has an antiproliferative effect on cancer cells in their examination of colorectal cancer cells.

Dose–response investigations indicate that BNP has no anticancer effects at any concentration (Gower et al. 2005, Vesely et al. 2005a,b, 2006a, 2007b,d). The addition of BNP for 24 h results in a 1, 2, and 4% (all non-significant) decrease in renal carcinoma cell numbers at its 1, 10, and 100 μM concentrations (Vesely et al. 2006c). CNP only has anticancer effects at 100-fold higher concentrations than that observed for the four cardiac hormones synthesized by the proANP gene (Vesely et al. 2005b, 2006c).

Cardiac hormones eliminate up to 80% of human pancreatic adenocarcinomas in vivo

Patients with human pancreatic adenocarcinomas have the lowest 5-year survival rate (1%) for all common types of cancers (Pitchumoni 1998, Wolff et al. 2010). The mean survival is only 4 months with pancreatic cancer (Pitchumoni 1998, Wolff et al. 2010). S.c. administration of the cardiac hormones for 28 days in athymic mice bearing human pancreatic adenocarcinomas results in ANP eliminating 80% of the human pancreatic carcinomas (Table 1; Vesely et al. 2007a,c). With each of the cardiac hormones, when human pancreatic adenocarcinomas are eliminated, they never recur in the primary site in the lifespan of mice (Vesely et al. 2007a,c). Metastatic lesions can also be eliminated by utilizing a different cardiac hormone from the one utilized to treat the primary lesion (Vesely et al. 2007a,c).

Table 1 Ability of the cardiac hormones to eliminate human cancers growing in athymic mice.

<table>
<thead>
<tr>
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<th>Breast cancer (%)</th>
<th>Pancreatic adenocarcinoma (%)</th>
<th>Small-cell lung cancer (%)</th>
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<tr>
<td>VDL</td>
<td>67</td>
<td>33</td>
<td>71</td>
</tr>
<tr>
<td>LANP</td>
<td>50</td>
<td>20</td>
<td>86</td>
</tr>
<tr>
<td>ANP</td>
<td>33</td>
<td>80</td>
<td>43</td>
</tr>
<tr>
<td>KP</td>
<td>67</td>
<td>14</td>
<td>57</td>
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The values are the percentages of human carcinomas eliminated and which never recur in the primary site in athymic mice when treated with each of the cardiac hormones for 28 days at 3 nM/kg body weight per min. VDL, vessel dilator; LANP, long-acting natriuretic peptide; ANP, atrial natriuretic peptide; KP, kaliuretic peptide.

Cardiac hormones eliminate up to 86% of human small-cell lung carcinomas in mice

The cardiac hormones can eliminate up to 86% of human small-cell lung carcinomas growing in athymic mice (Table 1; Eichelbaum et al. 2008). The treated small-cell lung carcinomas that are not eliminated grow rapidly, similar to the untreated controls. Metastatic small-cell lung cancers can be eliminated using these cardiac hormones in a sequential manner, one after the other, each for 4 weeks (Eichelbaum et al. 2008).

Cardiac hormones eliminate two-thirds of human breast carcinomas without any surgery

Vessel dilator and KP each eliminate 67% of human breast adenocarcinomas in athymic mice (Table 1) when infused subcutaneously for 28 days (Vesely et al. 2007b,d). There is no recurrence of breast cancer at the primary site in the 12-month post-treatment period (Vesely et al. 2007b,d). The respective abilities of the four cardiac hormones to eliminate different types of cancers vary with the type of cancer, as illustrated in Table 1.

Comparison of a twice-weekly i.v. treatment of pancreatic cancer for 4 weeks vs continuous treatment with ANP and vessel dilator for 4 weeks

To determine whether the cardiac hormones have beneficial effect(s) when given less frequently to mice with human pancreatic cancer, the animals were treated with vessel dilator or ANP twice weekly for 4 weeks with...
100 μM bolus infusions via a vascular port (Lenz et al. 2010). Vessel dilator with the biweekly bolus eliminated one-third of human pancreatic adenocarcinomas. Biweekly ANP eliminated one in six of human pancreatic adenocarcinomas in athymic mice (Lenz et al. 2010).

The marked difference between the biweekly infusions for 4 weeks (Lenz et al. 2010) and the continuous s.c. infusion of the cardiac hormones for 4 weeks (Vesely et al. 2007a,c) is that the biweekly treated pancreatic cancers that were not eliminated continued to grow and became very large as opposed to the continuous 28-day s.c. treatment with vessel dilator where the tumors that were not eliminated decreased in volume to 2–10% of that of untreated pancreatic carcinomas (Vesely et al. 2007a,c). This huge difference (P<0.0001) in the outcome of the treatment with the twice-weekly i.v. infusion for 4 weeks vs continuous s.c. infusions for 4 weeks suggest that the preferred treatment would be via s.c. infusion for 4 weeks for both cardiac hormones.

Mechanism of action of the cardiac hormones within cancer cells: receptors

The above-listed human cancer cells each have cardiac hormone receptors to mediate their effects (Gower et al. 2005, Vesely et al. 2005a,b,c, 2006c). Western blot analysis revealed the presence of the ANP receptors (NPR)-A (Fig. 2) and -C (Vesely et al. 2005c). NPR-A (Fig. 2) is an interesting receptor, since in addition to having a 441 a.a. binding site extending outside the cell membrane to bind ANP, it also has a protein kinase and guanylate cyclase as part of the receptor itself. Guanylate cyclase within the receptor catalyzes the formation of the intracellular mediator cyclic GMP which mediates the effects of these cardiac hormones within cancer cells as discussed below.

Metabolic targets of the cardiac hormones within cancer cells

RAS–MEK 1/2–ERK 1/2 kinase cascade

Inside the cancer cells, the cardiac hormones have multiple targets (Fig. 3). The cardiac hormones are multi-kinase inhibitors that inhibit the rat sarcoma-bound GTP (RAS)–mitogen-activated protein kinase kinase (MEK 1/2)–extracellular signal-related kinase (ERK 1/2) cascade pathway (Fig. 3; Sun et al. 2009a,b). This pathway is aberrantly activated in many types of neoplasms, with this activation being associated with a poor prognosis (Scholl et al. 2005, McCubrey et al. 2007, 2008). These cardiac hormones inhibit this pathway at several steps as follows.

RAS

RAS has frequent structural alterations in cancer cells, which makes RAS a difficult treatment target for cancer (McCubrey et al. 2007, Sebolt-Leopold 2008). Targeting RAS by interfering with son of sevenless gene (SOS) or growth factor receptor-bound 2 (GRB2; Fig. 3) has not yielded viable drug development candidates (Sebolt-Leopold 2008). Inhibitors of farnesyltransferase as a means of preventing the membrane localization of RAS have inhibited this prenylation enzyme; however, cancer cells have proven to be impervious to the action of this class of inhibitors (Sebolt-Leopold 2008). Thus, there is a need for a new agent(s) that can inhibit active RAS-GTP (Sebolt-Leopold 2008).

Vessel dilator and kaliuretic peptide inhibit the conversion of inactive RAS-GDP to active RAS-GTP by 95 and 90% respectively, with this inhibition lasting for 48–72 h (Sun et al. 2009a). Likewise, ANP and LANP inhibit the conversion of RAS-GDP to active RAS-GTP by 90 and 83% (Sun et al. 2009b). They inhibit the conversion to active RAS within 5 min (Sun et al. 2009b). With respect to what mediates their inhibitory action on RAS, it has been found that an antibody to cyclic GMP added with the four cardiac hormones and cyclic GMP itself inhibits RAS-GTP activation (up to 89%) (Sun et al. 2009a,b), suggesting that cyclic GMP mediates their inhibitory effects on RAS (Sun et al. 2009c, 2010). The four cardiac hormones also inhibit the stimulation of RAS by mitogens such as epidermal growth factor (EGF) and insulin.

MEK 1/2 kinases

The next step in the RAS–MEK 1/2–ERK 1/2 kinase cascade involves two kinases named MEK 1 and MEK 2 (Fig. 3). With respect to these two kinases, the protomer type, i.e. mitogen-activated protein kinase kinase (MKK-1) or MEK 1, specifically phosphorylates threonine and tyrosine residues present in the Thr-Glu-Tyr sequence of extracellular signal-regulated kinases 1 and 2 (ERK 1/2; Crews et al. 1992, Wu et al. 1993). A second MEK family member, i.e. MEK 2, resembles MEK 1 in phosphorylating ERK 1/2 but is seven residues longer than MEK 1 with its amino acid sequence being 81%, identical to MEK 1 (Wu et al. 1993). Vessel dilator, kaliuretic peptide, ANP, and LANP inhibit the phosphorylation of MEK 1/2 kinases (Fig. 3) by 98, 81, 88, and 97% respectively (Sun et al. 2007a,b).
The inhibition of MEK 1/2 kinases by the four cardiac hormones is maximal at 2 h (Sun et al. 2007a,b). With respect to what mediates their inhibitory effect, it has been found that MEK 1/2 kinases are also inhibited, as with RAS, by an antibody against cyclic GMP when added with the four cardiac hormones and cyclic GMP itself, inhibiting MEK 1/2 phosphorylation by 93% (Sun et al. 2007a,b).

**Inhibition of ERK 1/2 kinases**

ERK 1/2 are MEKs that are important targets for inhibiting the growth of cancer(s) (Davis 2000, Schlessinger 2000). Growth factors such as EGF and vascular endothelial growth factor (VEGF) mediate their cancer-causing effects via ERK 1/2 kinase activity (Schlessinger 2000). ERK 1/2 kinases can directly translocate to the nucleus and stimulate the production of several nuclear oncogenes such as c-fos (FOs; Davis 2000, Schlessinger 2000).

Vessel dilator, kaliuretic peptide, ANP, and LANP inhibit the phosphorylation of ERK 1/2 kinases by 96, 70, 94, and 88% (Sun et al. 2006a,b). Each has significant effects within 5 min and lasts for at least 2 h (Sun et al. 2006a,b). Thus, the cardiac hormones are multiple kinase inhibitors inhibiting the basal activity of each step in the RAS–MEK 1/2–ERK 1/2 kinase cascade in human cancer cells, as illustrated in Fig. 3.

**Mitogens such as EGF’s stimulation of RAS and ERK 1/2 kinases are also blocked by the cardiac hormones**

EGF has been shown to directly activate RAS (Kamada & Feramisco 1984, Satoh et al. 1990, Qui & Green 1991, Medema et al. 1992). Vessel dilator, LANP, ANP, and kaliuretic peptide reduce EGF-stimulated active RAS-GTP by 73, 79, 33, and 45% (Sun et al. 2010). The four cardiac hormones also inhibit up to 94% of the insulin-mediated conversion of RAS-GDP to active RAS-GTP (Sun et al. 2009c) that contributes to cancer formation (Ceresa & Pessin 1998).

EGF and insulin also stimulate ERK 1/2 kinases to cause cancer growth (Davis 2000, Schlessinger 2000). Insulin (1 μM) and EGF (10 ng/ml) each enhance the phosphorylation of ERK 1/2 kinases in pancreatic adenocarcinoma cells by 98 and 72% respectively (Sun et al. 2007c). This enhanced phosphorylation of ERK 1/2 by EGF and insulin is decreased up to 51% below non-stimulated (basal) ERK 1/2 activity by the four cardiac hormones (Sun et al. 2007c).

**Cardiac hormones’ ability to decrease the volume of pancreatic cancers and inhibit RAS, MEK 1/2, and ERK 1/2 kinases is mediated by the intracellular messenger cyclic GMP**

Cyclic GMP is the intracellular messenger of the cardiac hormones for some of their biological effects such as blood pressure regulation (Vesely 1977, Waldman et al. 1984). Evidence showing that cyclic GMP itself has strong anticancer effects is that cyclic GMP decreases the volume of human pancreatic cancer cells in vivo by 95% (Vesely et al. 2004).

Further evidence of cyclic GMP mediating the anticancer effects of the cardiac hormones suggests that a cyclic GMP antibody added to the cardiac hormones inhibits the ability of these hormones to block the basal activity of RAS (Sun et al. 2009a,b), MEK 1/2 (Sun et al. 2007a,b), and ERK 1/2 kinases (Sun et al. 2006a,b). Cyclic GMP itself inhibits the activation of RAS-GTP by 89% (Sun et al. 2009b), the phosphorylation of MEK 1/2 kinases by 93% (Sun et al. 2007b), and the phosphorylation of ERK 1/2 kinases by 83% (Sun et al. 2006b). Cyclic GMP, thus, appears to be very important for mediating the anticancer effects of these cardiac hormones in each step of the RAS–MEK 1/2–ERK 1/2 kinase cascade, as shown in Fig. 3.

**c-Jun N-terminal kinases**

c-Jun N-terminal kinase-2 (JNK) is associated with cancer development (Dann et al. 2001, Malbon 2004) and the invasion of cancers (Juneja et al. 2011). Lung cancer cell growth (Bost et al. 1997), prostate cancer proliferation, and prostate cancer growth are dependent upon JNK2 (Bost et al. 1999a,b, Yang et al. 2003). JNK-2 is activated by a variety of extracellular growth factors such as EGF (Kyriakas et al. 1994, Rosette & Karin 1996, Heasley & Han 2006). The activation of JNK by EGF is dependent upon H-RAS activation (Derijard et al. 1994, Bost et al. 1997). The loss of JNK activation coupled with the loss of ERK activation promotes cell death (Xia et al. 1995). As part of the cross-talk among these kinases, JNK is activated by MEK kinases (Fig. 3; Minden et al. 1994).

ANP and vessel dilator maximally reduce the expression of JNK2 by 89%, while LANP and kaliuretic peptide decrease JNK2 by 88 and 77% respectively in human small-cell lung cancer cells (Lane et al. 2012b). In human prostate adenocarcinoma cells, JNK2 was decreased by up to 84% by the four cardiac hormones (Lane et al. 2012b).
Vascular endothelial growth factor


The four cardiac hormones maximally decrease the VEGFR2 receptor in human pancreatic adenocarcinoma cells up to 83% (Nguyen et al. 2012). These four cardiac hormones decrease the VEGFR2 receptor by up to 89% in human small-cell lung cancer cells and up to 92% in human prostate cancer cells (Nguyen et al. 2012). These results were confirmed by Western blot that revealed a cardiac hormone-mediated decrease in VEGFR2 receptor (Nguyen et al. 2012). The cardiac hormones reduce VEGF concentrations up to 58% (Nguyen et al. 2012). Although there are a number of compounds that inhibit VEGF or its VEGFR2 receptor, the cardiac hormones are the first agents that are dual inhibitors of VEGF and its VEGFR2 receptor (Nguyen et al. 2012).

β-Catenin

One of the downstream targets of VEGF is β-catenin (Zhang et al. 2001). β-Catenin is a multifunctional protein located at the intracellular side of the cytoplasmic membrane that causes the malignant growth of colon (Mirabelli-Primidahl et al. 1999, Bienz & Clevers 2000), renal (Bilim et al. 2000, Maiti et al. 2000), and pancreatic (Lowe et al. 2003, Heiser et al. 2008) tumors. β-Catenin activation also leads to breast (Lin et al. 2000, Geyer et al. 2010), anaplastic thyroid (Garcia-Rostan et al. 1999, Abbosh & Nephew 2005), gastric (Ebert et al. 2003), liver (Thompson & Monga 2007), ovarian (Morin 1999), endometrial (Morin 1999), and prostate cancers (Voeller et al. 1998, Cheshire & Isaacs 2003). The gene that codes for β-catenin (CTNNB1) localizes to 3p21, a region implicated in tumor development (Kraus et al. 1994), which can also function as an oncogene (Wang et al. 2008).

These four cardiac hormones decrease the concentration of β-catenin up to 88% in human pancreatic cancer cells, up to 83% in human colorectal adenocarcinoma cells, and up to 73% in human renal adenocarcinoma cells (Skelton WP, IV, Skelton M & Vesely DL, 2012, unpublished observations). ANP induces a decrease in the expression of total β-catenin, which is associated with a redistribution of β-catenin from nuclear and cytoplasmic compartments to cell-to-cell junction sites and is associated with a decrease in the proliferation of colon adenocarcinoma cells (Serafino et al. 2012). Each of these cardiac hormones inhibits proliferation and can even inhibit the proliferation of cancer cells that have escaped cell death when treated with the cardiac hormones (Vesely et al. 2003, 2005a). ANP also causes a significant down-regulation of c-Myc (MYC) and cyclin D-1 gene transcriptions regulated by β-catenin (Serafino et al. 2012). β-Catenin appears to be the central target of the anticancer effects of the cardiac hormones since these hormones inhibit upstream RAS kinase, which activates β-catenin (Abbosh & Nephew 2005), and downstream c-Jun N-terminal kinase and VEGF, which are activated by β-catenin, as illustrated in Fig. 3 (Mann et al. 1999, Zhang et al. 2001).

WNT signaling pathway

The WNT signaling pathway is a signal transduction pathway that is enhanced in a variety of cancers (Bienz & Clevers 2000, Polakis 2012). The origin of the name WNT comes from a portmanteau of Int (integration 1 gene in breast cancer) and Wg (wingless) in Drosophila, which has the best characterized WNT gene (Polakis 2012). The WNT gene family encodes for several signaling proteins, of which WNT-3a is a protein encoded in humans (Katoh 2002). WNT signaling is stimulated by RAS (Li et al. 2005) and VEGF pathways (Li et al. 2005). Both RAS and VEGF contribute to the pathobiology of colon cancer, in part, through the WNT pathway (Okada et al. 1998). KRAS and WNT pathways can cooperate to regulate the VEGF gene (Zhang et al. 2001). In human pancreatic carcinoma cells, WNT-3a maximally decreases 68% secondary to the four cardiac hormones (Skelton et al. 2013c). The four cardiac hormones also maximally reduce the concentration of WNT-3a by up to 53% in human colorectal adenocarcinoma cells (Skelton et al. 2013c). The complex interplay of WNT, RAS, and VEGF in causing cancer and maintaining its growth (Okada et al. 1998, Bienz & Clevers 2000, Zhang et al. 2001, Katoh 2002, Li et al. 2005, Polakis 2012) is all targeted by the four cardiac hormones, which helps to explain their dramatic effects of eliminating up to 86% of human cancers growing in...
mice and the fact that, once eliminated, these human cancers never return to the primary site in the lifespan of mice (Vesely et al. 2007a,b,c,d, Eichelaum et al. 2008).

**Secreted frizzled-related protein-3**

Secreted frizzled-related protein-3 (sFRP-3) is an ~300 a.a. glycoprotein (Lin et al. 1997, Rattner et al. 1997, Dann et al. 2001, Malbon 2004) that promotes renal cancer growth when injected into athymic mice (Hirata et al. 2001, Malbon 2004) that promotes renal cancer growth. sFRP-3 has been linked to tumor promotion in other types of cancers as well (Rubin et al. 2006). Bovolenta et al. (2008) have suggested that elevated sFRPs in various types of cancers may be a viable therapeutic target. ANP has effects through a frizzled-receptor which contains sFRP-3 (Xu & Nusse 1998, Kawano & Kypta 2003) mediated activation (Serafino et al. 2012). ANP and the frizzled receptor co-localize on the cell membrane within 30 min after ANP addition to culture medium (Serafino et al. 2012). We have found that vessel dilator, kaliuretic peptide, ANP, and LANP decrease the levels of sFRP-3 by 77–78% in human pancreatic cancer cells, 83–84% in human colorectal cancer cells, and 66–68% in human renal cancer cells (Skelton et al. 2013a). With respect to the mechanism by which the reduction of sFRP-3 levels by the cardiac hormones leads to their anticancer effects, their ability to inhibit sFRP-3, the active cysteine-rich domain (CRD) of the frizzled receptor (Rattner et al. 1997), blocks the propagation of the signal responsible for causing cancer cell growth.

**AKT**

AKT, also known as protein kinase B (PKB), is a serine/threonine protein kinase that has a key role in cell proliferation and in the growth of many types of cancer (Vivanco & Sawyer 2002, Altomare & Testa 2005, Hay 2005, Hennessy et al. 2005, Shaw & Cantley 2006). The name AKT derives from the ‘Ak’ mouse strain that develops spontaneous thymic lymphomas, where ‘t’ stands for thymoma (Staal et al. 2007). AKT is overexpressed in colorectal cancer cells but not in normal colonic mucosa and hyperplastic polyps (Roy et al. 2002). ANP decreases the activation of AKT about twofold between 2 and 4 h of treatment in cell culture (Serafino et al. 2012). The other cardiac hormones also decrease AKT (Skelton et al. 2013b) with their results as follows: vessel dilator, kaliuretic peptide, and LANP reduce the concentration of AKT by 47, 45, and 46% in human colorectal cancer cells, by 60, 61, and 59% in human pancreatic carcinoma cells, and by 31, 32, and 31% in renal adenocarcinoma cells. There is a complex interplay of AKT, RAS, and VEGF in causing cancer and maintaining cancer cell growth (Gerber et al. 1998, Zhang et al. 2001, Vivanco & Sawyer 2002, Altomare & Testa 2005, Amaravadi & Thompson 2005). This interplay is modified (inhibited) by these four cardiac hormones. There is a cross-talk between the activation of AKT and its inhibition by the cardiac hormones, which is summarized as follows: RAS activates AKT (Crowell et al. 2007). Growth factors such as EGF also activate RAS with a resultant downstream activation of AKT (Crowell et al. 2007). The effects of VEGFs on cancer growth and metastasis are mediated by binding to the VEGFR2 (KDR/Flik-1) receptor, which, in turn, activates the AKT pathway (Gerber et al. 1998). The four cardiac hormones inhibit each of these steps.

**STAT**

STATs are cytoplasmic transcription factors (Schindler et al. 1992, Yu & Jove 2004) which are the final ‘switches’ that activate gene expression patterns that lead to malignancy (Schindler et al. 1992, Darnell 2002, Yu & Jove 2004). STAT3 of the STATs is important in cancer formation (Bromberg & Darnell 2002, Yu & Jove 2004). STAT3 is overexpressed in a variety of human tumors and therefore could be a target for cancer treatment (Grandis et al. 1998, Song et al. 2003, Yu & Jove 2004). The EGF receptor-mediated growth of squamous carcinoma cells is known to require STAT3 but not STAT1 (Grandis et al. 1998). Targeting STAT3 is also a strategy for reversing paclitaxel therapy resistance (Duan et al. 2006).

ERK 1/2 activates (i.e. phosphorylates) STAT3 at serine 727 in response to growth factors (Chung et al. 1997). STAT3 is an excellent substrate for ERK kinases (Chung et al. 1997) and, as above, the cardiac hormones each inhibit ERK 1/2 kinases. Vessel dilator, LANP, kaliuretic peptide, and ANP decrease STAT3 by 88, 54, 55, and 65% respectively in human small-cell lung cancers, and by 66, 57, 70, and 77% in human pancreatic adenocarcinoma cells (Lane et al. 2012a). These cardiac hormones do not decrease STAT1 in either human small-cell lung cancer or pancreatic adenocarcinoma cells (Lane et al. 2012a). Thus, the four cardiac hormones are significant inhibitors of STAT3 but sparing STAT1, which suggests a specificity for the anticancer mechanism(s) of action of these hormones in human cancer cells (Lane et al. 2012a).
Four cardiac hormones cause cytotoxicity of human cancer cells but not of healthy cells

One would surmise that the cardiac hormones may be cytotoxic rather than cytostatic, as cytostatic agents do not cause tumor shrinkage or elimination of cancers as the cardiac hormones do (Pitchumoni 1998, Vesely et al. 2007b, d, Eichelbaum et al. 2008). Cytotoxicity secondary to the cardiac hormones has been directly tested with a Cyto-Tox-Glo Cytotoxicity Assay (Promega), which is a cell-based luminescent assay that measures the extracellular activity of a distinct intracellular protease (dead-cell protease) when this protease is released from membrane-compromised cells (Niles et al. 2007). The results of this assay directly correlate with the percentage of cells undergoing cytotoxicity (Niles et al. 2007). The four cardiac hormones have been found to cause cytotoxicity of up to 75% of human prostate cancer cells (Pi et al. 2011). There was no cytotoxicity of prostate and lung cells from healthy individuals exposed to the same concentrations of the cardiac hormones for an identical length of time (Pi et al. 2011). Thus, the four cardiac hormones cause cytotoxicity in human cancer cells while sparing healthy human cells.

Four cardiac hormones cause cell death of human cancer cells but not of healthy cells

Nuclear matrix proteins (NMPs) make up the internal structure (framework) of the nucleus and are associated with RNA synthesis (Hancock & Boulikas 1982, Bouteille et al. 1983). Cell death releases soluble NMPs that can be detected in culture supernatant and other fluids containing dead and dying cells (Berrios et al. 1985, Zeitlin et al. 1987), and their measurement is useful to quantify cell death (Bouteille et al. 1983).

The cardiac hormones cause cell death in up to 36% of pancreatic adenocarcinoma cells and in up to 28% of prostate cancer cells over a concentration range of 100 pM–10 μM as quantified by measuring NMP 4117, which is a function of the number of dead or dying cells (Skelton et al. 2012). There was no cell death of healthy human prostate, kidney, or lung cells at the above concentrations (Skelton et al. 2012). Thus, these four cardiac hormones that cause the death of cancer cells spare healthy human prostate, lung, or kidney cells from cell death.

Declaration of interest
The patent to treat cancer with the cardiac hormones has been assigned to the University of South Florida, which has not licensed this patent to any commercial entity. There has been no pharmaceutical company funding or input into the studies described herein.

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Dr Vesely is the sole author of this review.

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