Abstract. The heart is a sophisticated endocrine gland synthesizing the atrial natriuretic peptide (ANP) prohormone which contains four peptide hormones, namely atrial natriuretic peptide, vessel dilator, kaliuretic peptide and long-acting natriuretic peptide, which decrease up to 97% of human pancreatic, breast, colon, prostate, kidney and ovarian carcinomas, as well as small-cell and squamous cell lung cancer cells within 24 hours in cell culture. In vivo these four cardiac hormones eliminate up to 80% of human pancreatic adenocarcinomas, up to two-thirds of human breast cancers, and up to 86% of human small-cell lung cancers in athymic mice. Their anticancer mechanism(s) target the Rat sarcoma bound guanosine triphosphate (RAS)-mitogen activated protein kinase kinase 1/2 (MEK1/2)-extracellular signal related kinase 1/2 (ERK1/2) kinase cascade in cancer cells. These four cardiac hormones inhibit up to 95% of the basal activity of Ras, 98% of the phosphorylation of MEK1/2 kinases and 96% of the activation of basal activity of ERK1/2 kinases. They also completely block the activity of mitogens such as the ability of epidermal growth factor to stimulate ERK and RAS. In addition to inhibiting these mitogen-activated protein kinases (MAPKs) they also inhibit MAPK9, i.e. c-Jun-N-terminal kinase 2. These multiple kinase inhibitors are cytotoxic and cause cell death of cancer cells but not of normal cells.

The heart is a sophisticated endocrine gland synthesizing a 126 amino acid (a.a.) prohormone which contains four peptide hormones (1-3). These peptide hormones are involved in blood pressure regulation and maintenance of plasma volume in animals (4-9) and humans (10-12). These cardiac hormones, numbered by their a.a. sequences beginning at the N-terminal end of the atrial natriuretic peptide (ANP) prohormone, consist of the first 30 a.a. of the prohormone, namely, long-acting natriuretic peptide (LANP), a.a. 31-67 (namely, vessel dilator), a.a. 79-98 (kaliuretic peptide) and a.a. 99-126 (ANP) (13, 14). Each of these peptide hormones circulates in healthy humans, with the concentrations of vessel dilator and LANP in plasma being 17- to 24-fold higher than that of ANP (15-20).

Heart Hormones Eliminate up to 97% of Cancer Cells In Vitro

The four cardiac hormones reduce up to 97% of human pancreatic, colon, prostate, breast, ovarian and kidney adenocarcinoma cells (21-26), angiosarcoma of the heart cells (27), melanomas (28), medullary thyroid carcinomas (29), glioblastomas of the brain (30), as well as small-cell (31) and squamous cell lung carcinoma cells (32) in cell culture. There is a 97.4%, 87%, 88% and 89% (p<0.0001 for each) decrease (namely, elimination) of human prostate adenocarcinoma cells by vessel dilator, LANP, kaliuretic peptide, and ANP, respectively, within 24 hours at their 1 mM concentrations (23). There is no proliferation in the three days following this decrease, induced by the heart hormones (23). When antibodies to these four cardiac hormones are used, the ability of the hormones to reduce the number of prostate cancer cells is completely blocked, indicating that their effects are specific, namely not due to some other hormone or substance (23). Dose response curves have revealed that there is a significantly greater (p<0.05) decrease in the number of cancer cells at each 10-fold increase in the concentration of the four cardiac hormones synthesized by the ANP gene in human breast, colon and prostate cancer cells as well as in small-cell and squamous cell carcinoma of lung cells (21-26, 31, 32).
Heart Hormones Eliminate up to 80% of Human Pancreatic Adenocarcinomas In Vivo

Human pancreatic adenocarcinoma has the lowest 5-year survival rate of all common types of cancers (33, 34). The 5-year survival rate of patients with adenocarcinoma of the pancreas is 1%, with a median survival of only four months (33, 34). Current cancer chemotherapy and surgery prolong survival by a few months, but the mean survival of four months, refers to patients who were treated with surgery and/or current cancer chemotherapeutic agents (33, 34).

When each of the heart hormones are infused subcutaneously at 3 nM min⁻¹ kg⁻¹ body weight for 28 days in athymic mice bearing human pancreatic adenocarcinomas, ANP eliminated 80% of the human pancreatic carcinomas (35). Vessel dilator, LANP and KP eliminated the primary pancreatic carcinomas by 33%, 20%, and 14% in their respective treatment groups (35). In none of the animals in which the pancreatic adenocarcinomas are eliminated in the primary site, does a single animal ever have a recurrence in the primary site (35). One ANP-treated animal developed a metastatic lesion and this lesion was eliminated by treatment with vessel dilator (35). Even in the treated animals which did not have total elimination of their human pancreatic adenocarcinoma with the four respective heart hormones, tumor volumes decreased to less than 10% (and with vessel dilator to less than 2%) compared to those of the untreated animals, both during treatment and in a 12-month follow-up period (35).

Table I. Ability of cardiac hormones to eliminate human cancer growing in athymic mice. The numbers in each column are the percentages of human carcinomas eliminated and which never recurred in the primary site in athymic mice, when treated with each of the cardiac hormones for 28 days at 3 nM/kg body weight/minute. VDL, vessel dilator; LANP, long-acting natriuretic peptide; ANP, atrial natriuretic peptide; KP, kaliuretic peptide.

<table>
<thead>
<tr>
<th>Cancer Type</th>
<th>VDL</th>
<th>LANP</th>
<th>ANP</th>
<th>KP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Breast adenocarcinoma</td>
<td>67%</td>
<td>50%</td>
<td>33%</td>
<td>67%</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>33%</td>
<td>20%</td>
<td>80%</td>
<td>14%</td>
</tr>
<tr>
<td>Small-cell lung cancer</td>
<td>71%</td>
<td>43%</td>
<td>43%</td>
<td>57%</td>
</tr>
</tbody>
</table>

Heart and Kidney Hormones Eliminate up to 86% of Human Small-Cell Lung Carcinomas in Mice

LANP, vessel dilator, KP, and ANP eliminate 86%, 71%, 57%, and 43% (p<0.001 for each) of human small-cell lung carcinomas (36). Treated small-cell lung carcinomas that are not eliminated grow rapidly, similar to the untreated controls, whose volume is 7-fold larger in one week, 18-fold increased in two weeks, 39-fold increased in three weeks, 63-fold increased in one month and 97-fold increased in volume in six weeks (36). One vessel dilator-treated animal with small-cell lung carcinoma developed a large tumor (8,428 mm³ volume) upon treatment and this tumor was eliminated utilizing ANP and then LANP, each for four weeks sequentially (36).

Heart Hormones Eliminate Two-Thirds of Human Breast Carcinomas without any Surgery

Vessel dilator, LANP, KP and ANP eliminate 67%, 50%, 67% and 33% of the human breast adenocarcinomas in athymic mice, when infused subcutaneously for 28 days, with fresh hormones prepared weekly at 3 nM min⁻¹ kg⁻¹ body weight (37). There was no recurrence of the breast cancer at the primary site and no metastasis, except in the ANP-treated group (37). The natriuretic peptide receptors A and C were decreased 50% and 31%, respectively, in metastatic versus primary ANP-treated breast adenocarcinomas, possibly explaining why fewer of the breast carcinomas responded to ANP compared to the other three cardiac hormones, as ANP works via these receptors while the other peptide hormones have their own specific receptors (2, 3, 37). Thus, the four hormones made within the heart have the ability to eliminate human pancreatic, breast and small-cell lung cancer growing in mice but their respective abilities to eliminate these...
different types of cancers varies with the type of cancer, as illustrated in Table I.

**Mechanism of Action of Heart Hormones: Receptors**

Each of the human cancer cell types examined above has natriuretic peptide receptors to mediate the effects of these peptide hormones (21-32). Thus, when human breast and kidney adenocarcinoma cells were evaluated by western blots, natriuretic peptide receptors (NPR) A, B and C found to be present (22-25). Breast adenocarcinoma cells have developed NPR-A and -C receptors to mediate the effects of ANP via a membrane-bound guanylate cyclase, which is part of the NPR-A receptor, and via NPR-C receptor-mediated mechanisms, respectively (2, 3). The NPR-C receptor does not contain guanylate cyclase, which catalyzes the formation of the intracellular messenger cyclic GMP (2, 3).

**Metabolic Targets of Heart Hormones within Cancer Cells**

After binding to their respective receptors on the cancer cells, the cardiac hormones inhibit the RAS mitogen-activated protein kinase (MAPK)/extracellular signal-related kinase-kinase (MEK)-ERK1/2 kinase pathway (Figure 2), which is a prototypical signal transduction pathway in cancer (38, 39). This pathway is aberrantly activated in many types of neoplasms, including prostate and breast cancer, with this activation being associated with a poor prognosis (38, 39). These heart hormones inhibit this pathway at several steps (namely, multiple kinase inhibitors), as follows.

**RAS.** Structural alteration in the GTPase RAS occurs in 25% to 30% of human carcinomas, which allows them to relay mitogen signals in a ligand-independent manner, thereby obviating the need for ligand activation of growth factor
receptors that occurs in normal cells (40, 41). Attempts to target RAS by perturbing its interaction with either Son of Sevenless gene (SOS) or with growth factor receptor-bound 2 (GRB2) (Figure 2), have not yielded viable drug development candidates, largely because of the inherent difficulties in disrupting protein–protein interactions with drug-like molecules (40). Several drug discovery programs have also been devoted to finding inhibitors of farnesyltransferase as a means of preventing the membrane localization of RAS (40). Despite the successful identification of several chemical leads that effectively inhibited this prenylation enzyme, tumor cells, have generally been proven to be impervious to the action of this class of inhibitors (40).

Vessel dilator and kaliuretic peptide (each at 1 μM) inhibited the phosphorylation of RAS by 95% and 90% \((p<0.0001)\), respectively. At 0.01 μM of kaliuretic peptide, the maximal inhibition was 95% \((p<0.0001)\). The inhibition of RAS lasted for 48 to 72 hours, secondary to both peptides (42). Their ability to inhibit RAS was inhibited by an antibody against cyclic GMP, and cyclic GMP itself inhibited RAS phosphorylation \((89%; p=0.0015)\) (42).

ANP and LANP (each at 0.1 μM) inhibited the phosphorylation of RAS by 90% and 83% \((p<0.0001)\) (43). At 0.01 μM of LANP, the maximal inhibition was 89%, which occurred within 5 minutes. Both peptide hormones inhibit RAS for 3 to 4 hours (43). Their ability to inhibit RAS is also inhibited by an antibody against cyclic GMP and cyclic GMP itself inhibits RAS phosphorylation \((72%; p=0.009)\) (43). Stimulation of RAS by mitogens such as epidermal growth factor (EGF) is also inhibited by these four cardiac hormones (44).

**MEK1/2 kinases.** Vessel dilator and kaliuretic peptide (each at 10 μM) inhibited the phosphorylation of MEK1/2 kinase by 98% and 81% \((p<0.0001)\) (Figure 1; (45)). The inhibition of MEK1/2 lasted for at least two hours, when it was maximal, with both heart hormones (45).

LANP and ANP (each at 10 μM) inhibited the phosphorylation of MEK1/2 kinase by 97% and 88% \((p<0.00001)\) (46). The inhibition of MEK1/2 was maximal at two hours and ceased after four hours with both heart hormones (46). The ability of the heart hormones to inhibit MEK1/2 is also inhibited by an antibody against cyclic GMP and cyclic GMP itself inhibited MEK1/2 phosphorylation by 93% (46). Thus, ANP, vessel dilator, KP and LANP each inhibit MEK1/2 kinase mediated via cyclic GMP, as part of their anticancer mechanism(s) of action (45, 46).

**Inhibition of the activation of ERK 1/2 kinases.** Vessel dilator and kaliuretic peptide (each at 1 μM) inhibited the activation, namely the phosphorylation, of ERK1/2 kinases by 96% and 70% \((p<0.0001)\) (47). Both have significant effects within five minutes at a concentration of 0.01 μM (47). The inhibition of ERK1/2 lasted for at least two hours with both heart hormones (47). Maximal inhibition of the phosphorylation of ERK1/2 kinases by ANP and LANP was
94% and 88%, respectively (p<0.0001) (48). The inhibition of ERK1/2 kinases lasted for at least two hours, when it was maximal, with ANP and LANP (48).

c-Jun-N-terminal kinases. c-Jun-N-terminal kinase (JNK), also known as stress-activated protein kinase 1 alpha (SAPK1α) is activated by a variety of extracellular stimuli such as growth factors and environmental stresses (49, 50). Thus, JNK is activated by EGF, tumor necrosis factor, platelet-derived growth factor and transforming growth factor, as well as by diverse environmental stresses (51-53). Activation of JNK by EGF is dependent upon H-RAS activation (51, 54). JNK2 is not only associated with cancer development but also with invasion of cancer, *e.g.* breast cancer (55). Loss of JNK activation coupled with loss of ERK activation promotes cell death (56). Lung cancer cell growth (53), prostate cancer proliferation and prostate cancer xenograft growth are dependent upon JNK2 (57, 58). Of all the JNK kinases, it is JNK2, also known as mitogen-activated protein kinase 9 (MAPK9), that is preferentially required for mediating proliferation of lung cancer (59) and prostate cancer cells (57).

Vessel dilator, LANP, kaliuretic peptide and ANP maximally reduced the expression of JNK2 by 89%, 88%, 77%, and 89%, respectively (each at p<0.0001), in human small-cell lung cancer cells (60). In human prostate adenocarcinoma cells, JNK2 was maximally decreased by 76%, 84%, 57%, (each at p<0.0001), and 26% (p<0.01) by vessel dilator, LANP, kaliuretic peptide, and ANP, respectively (60). These results indicate that the four cardiac hormones are significant inhibitors (by up to 89%) of JNK2 in human small-cell lung cancer cells and in human prostate adenocarcinoma cells (up to 84%) as part of their anticancer mechanism(s) of action.

**Four Heart Hormones Cause Cytotoxicity of Human Cancer Cells But Not of Healthy Cells**

It would be expected that the heart hormones may be cytotoxic rather than cytostatic as cytostatic agents do not cause tumor shrinkage or elimination as these heart hormones do (33, 36, 37). Cytotoxicity was directly tested with a Cyto-Tox-Glo™ Cytotoxicity Assay (Promega, Madden, WI, USA), which is a cell-based luminescent assay that measures the extracellular activity of a distinct intracellular protease (dead-cell protease) when the protease is released from membrane-compromised cells (61). The results of this assay directly correlate with the percentage of cells undergoing cytotoxicity (61). Over a seven-day period, the four heart hormones caused cytotoxicity of up to 75% on human prostate cancer cells and up to 58% on human pancreatic cancer cells, over a concentration range of 100 pM to 1 μM (62). There was no cytotoxicity towards prostate and lung cells from healthy individuals exposed to the same concentrations of the cardiac hormones for seven days (62).

This study demonstrates that four cardiac hormones cause cytotoxicity in human cancer cells while sparing healthy human cells, when evaluated over a seven-day period.

**Four Heart 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 such functions as DNA replications, RNA synthesis, and hormone receptor binding (63, 64). The identification of cell type-specific NMPs supports their potential contribution to cellular differentiation and tissue development (64). Although the nuclear matrix has been shown to be highly insoluble *in vitro*, it is now known that cell death releases soluble NMPs that can be detected in the culture supernatant and other fluids containing dead and dying cells (65, 66). Because the level of NMP 41/7 detected in the culture supernatant is a function of the number of dead or dying cells, the measurement of NMP 41/7 is useful in quantifying cell death (65).

Each of these heart hormones caused death in up to 36% (p<0.0001) of pancreatic adenocarcinoma cells and in up to 28% (p<0.0001) of prostate cancer cells, over a concentration range of 100 pM to 10 μM (67). There was no cell death of normal human prostate, kidney, or lung cells at the above concentrations (67). Thus, these four cardiac hormones cause death of pancreatic and prostate cancer cells but, at the same concentrations, they do not eliminate normal prostate, lung, or kidney cells.

**Conclusion**

Four cardiac hormones coded for by one gene (namely, atrial natriuretic peptide) have significant (p<0.0001) anticancer effects in reducing up to 97% of all cancer cells tested in cell culture (21-32). Furthermore, these cardiac hormones eliminate up to 80% of human pancreatic adenocarcinomas, up to two-thirds of human breast carcinomas and up to 86% of human small-cell lung carcinomas in athymic mice, suggesting that they may have very beneficial effects on a number of different types of cancer (35-37). Their mechanism(s) of action towards cancer cells is a very strong (up to 98%) inhibition of the activity of the RAS-MEK1/2-ERK1/2 kinase cascade mediated *via* the intracellular mediator cyclic GMP (42-48). Further clinical studies should determine their utility in humans with cancer.

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