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.

Key Words

- cancer
- hormones
- RAS
- β -catenin

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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

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 (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 2006a Vessel dilator and kaliuretic peptide inhibit activation of ERK 1/2 in human prostate cancer cells. *Anticancer Research* 26 3217–3222.

long-acting natriuretic peptide (LANP) that consists of the first 30 a.a. from the N-terminal end of the ANP 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 (Vesely 1992, 2002; Fig. 1). The BNP and CNP genes, on the other hand, appear to each synthesize only one peptide hormone within their respective hormones, namely BNP and CNP (Gardner *et al.* 1997, Lainchbury *et al.* 1997, Scotland *et al.* 2005). Each of these peptide hormones circulates in humans and animals (Vesely *et al.* 1989, Winters *et al.* 1989, Hunter *et al.* 1998, De Palo *et al.* 2000, Franz *et al.* 2000, 2001, Vesely 2003, Cataliotti & Burnett 2005). When these four cardiac hormones synthesized by the ANP prohormone gene are given in concentrations higher than those synthesized by the heart, they have anticancer effects on human pancreatic cancers growing in athymic mice (Vesely *et al.* 2007a,c). BNP does not appear to have any anticancer effects (Gower *et al.* 2005, Vesely *et al.* 2005a,b, 2006c, 2007b,d). CNP has anticancer effects *in vitro* but only when given in 100-fold higher concentrations than the above four cardiac hormones synthesized by the ANP prohormone gene (Vesely *et al.* 2005b, 2006c). This review will concentrate on the four cardiac hormones from the ANP prohormone and their anticancer effects.

All of the effects of the cardiac hormones on cancer appear to be mediated via the intracellular messenger cyclic GMP formed by enhancing the enzyme guanylate cyclase, which is part of natriuretic peptide receptor-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).

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

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 the intracellular messenger 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 *Atrial Natriuretic Hormones*, pp 1–256. Englewood Cliffs, New Jersey: Prentice Hall.

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.

receptor does to increase cyclic GMP (Vesely 1992). 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 E_2 (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 E_2 (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 E_2 , which is synthesized secondary to vessel dilator and kaliuretic peptide (Gunning *et al.* 1992, Chiou & Vesely 1995). The ability of prostaglandin E_2 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.

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, 2006c, 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 μ M 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

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

	Breast cancer (%)	Pancreatic adenocarcinoma (%)	Small-cell lung cancer (%)
VDL	67	33	71
LANP	50	20	86
ANP	33	80	43
KP	67	14	57

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.

the one utilized to treat the primary lesion (Vesely *et al.* 2007a,c).

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 μ M 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 prototype member, 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 μ M) 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

VEGF plays an essential role throughout tumor development by enabling the blood vessels to establish and grow into tumors, thereby providing nutrients and oxygen to the tumor (Folkman 1971, 2007, Liotta *et al.* 1974, Ferrara 2004, Hoeben *et al.* 2004). VEGF intracellularly enables cancer cells to grow via stimulating RAS (Doanes *et al.* 1999, Meadows *et al.* 2001), MEK 1/2 (Byrne *et al.* 2005, Wang *et al.* 2009), and ERK 1/2 kinases (Gupta *et al.* 1999, Breslin *et al.* 2003). VEGFR2/KDR/Flk-1 is the main VEGF receptor mediating the cancer-enhancing effects of VEGF (McMahon 2000, Ferrara 2004, Hoeben *et al.* 2004). The cardiac hormones are dual inhibitors of VEGF and its receptor VEGFR2 as outlined below.

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, Eichelbaum *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.* 2010). 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 & Sawyers 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 & Sawyers 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/Flk-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 μ M 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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Author contribution statement

Dr Vesely is the sole author of this review.

References

- Abbosh PH & Nephew KP 2005 Multiple signaling pathways converge on β -catenin in thyroid cancer. *Thyroid* **15** 551–561. (doi:10.1089/thy.2005.15.551)
- Altomare DA & Testa JR 2005 Perturbations of the AKT signaling pathway in human cancer. *Nature Reviews. Cancer* **24** 7455–7464.
- Amaravadi R & Thompson CB 2005 The survival kinases AKT and Pim as potential pharmacological targets. *Journal of Clinical Investigation* **115** 2618–2624. (doi:10.1172/JCI26273)
- Benjamin BA & Peterson TV 1995 Effects of ProANF (31–67) on sodium excretion in conscious monkeys. *American Journal of Physiology* **269** R1351–R1355.
- Berrios M, Osheroff N & Fisher PA 1985 *In situ* localization of DNA topoisomerase II, a major polypeptide component of the *Drosophila* nuclear matrix fraction. *PNAS* **82** 4142–4146. (doi:10.1073/pnas.82.12.4142)
- Bienz M & Clevers H 2000 Linking colorectal cancer to Wnt signaling. *Cell* **103** 311–320. (doi:10.1016/S0092-8674(00)00122-7)
- Bilim V, Kawasaki T, Katagiri A, Wakatsuki S, Takahashi K & Tomita Y 2000 Altered expression of β -catenin in renal cell cancer and transitional cell cancer with the absence of β -catenin gene mutations. *Clinical Cancer Research* **6** 460–466.
- Bost F, Dean N, McKay R & Mercola D 1997 Activation of the Jun kinase/stress-activated protein kinase pathway is required for EGF-autocrine stimulated growth of human A549 lung carcinoma cells. *Journal of Biological Chemistry* **272** 33422–33429. (doi:10.1074/jbc.272.52.33422)
- Bost F, Potapova O, Liu C, Zhang YM, Charbono W, Dean N, McKay R & Mercola D 1999a High frequency regression of established human prostate carcinoma PC3 xenografts by systemic treatment with antisense Jun kinase. *Prostate* **38** 320–321.
- Bost F, McKay R, Bost M, Potapova O, Dean NM & Mercola D 1999b Jun kinase-2 isoform is preferentially required for epidermal growth factor-induced proliferation of human A549 lung carcinoma. *Molecular and Cellular Biology* **19** 1938–1949.
- Bouteille M, Bouvier D & Seve AP 1983 Heterogeneity and territorial organization of the nuclear matrix and related structures. *International Review of Cytology* **83** 135–182.
- Bovolenta P, Esteve P, Ruiz JM, Cisneros E & Lopez-Rios J 2008 Beyond WNT inhibition: new functions of secreted frizzled-related proteins in development and disease. *Journal of Cell Science* **121** 737–746. (doi:10.1242/jcs.026096)
- Brenner BM, Ballermann BJ, Gunning ME & Zeidel ML 1990 Diverse biological actions of atrial natriuretic peptide. *Physiological Reviews* **70** 665–699.
- Breslin JW, Pappas PJ, Cerveira JJ, Hobson RW, II & Duran WN 2003 VEGF increases endothelial permeability by separate signaling pathways involving ERK 1/2 and nitric oxide. *American Journal of Physiology* **284** H92–H100.

- Bromberg J & Darnell JE, Jr 2002 The role of STATs in transcriptional control and their impact on cellular function. *Oncogene* **19** 2468–2473. (doi:10.1038/sj.onc.1203476)
- Byrne AM, Bouchier-Hayes DJ & Harmey JH 2005 Angiogenic and cell survival functions of vascular endothelial growth factor (VEGF). *Journal of Cellular and Molecular Medicine* **9** 777–794. (doi:10.1111/j.1582-4934.2005.tb00379.x)
- Cataliotti A & Burnett JC, Jr 2005 Natriuretic peptides: novel therapeutic targets in heart failure. *Journal of Investigative Medicine* **53** 378–384. (doi:10.2310/6650.2005.53711)
- Ceresa BP & Pessin JE 1998 Insulin regulation of the RAS activation/inactivation cycle. *Molecular and Cellular Biochemistry* **182** 23–29. (doi:10.1023/A:1006819008507)
- Cheshire DR & Isaacs WB 2003 β -Catenin signaling prostate cancer: an early perspective. *Endocrine-Related Cancer* **10** 537–560. (doi:10.1677/erc.0.0100537)
- Chiou S & Vesely DL 1995 Kaliuretic peptide: the most potent inhibitor of Na^+/K^+ -ATPase of the atrial natriuretic peptides. *Endocrinology* **136** 2033–2039. (doi:10.1210/en.136.5.2033)
- Chung J, Uchida E, Grammer TC & Blenis J 1997 STAT3 serine phosphorylation by ERK-dependent and -independent pathways negatively modulates its tyrosine phosphorylation. *Molecular and Cellular Biology* **17** 6508–6516.
- Crews CM, Alessandrini A & Erikson RL 1992 The primary structure of MEK, a protein kinase that phosphorylates the ERK gene product. *Science* **258** 478–480. (doi:10.1126/science.1411546)
- Crowell JA, Steele VE & Fay JR 2007 Targeting the AKT protein kinase for cancer chemoprevention. *Molecular Cancer Therapeutics* **6** 2139–2148. (doi:10.1158/1535-7163.MCT-07-0120)
- Dann CE, Hsieh JC, Rattner A, Sharma D, Nathans J & Leahy DJ 2001 Insights into WNT binding and signaling from the structures of two frizzled cysteine-rich domains. *Nature* **412** 86–90. (doi:10.1038/35083601)
- Darnell JE, Jr 2002 Transcription factors as targets for cancer therapy. *Nature Reviews. Cancer* **2** 740–749. (doi:10.1038/nrc906)
- Davis RJ 2000 Signal transduction by the JNK group of MAP kinases. *Cell* **103** 239–252. (doi:10.1016/S0092-8674(00)00116-1)
- De Palo EF, Woloszczuk W, Meneghetti M, De Palo CB, Nielsen HB & Secher NH 2000 Circulating immunoreactive proANP (1–30) and proANP (31–67) in sedentary subjects and athletes. *Clinical Chemistry* **46** 843–847.
- Derijard R, Hibi M, Wu IH, Barrett T, Su B, Dent TL, Karin M & David RJ 1994 JNK1-a protein kinase stimulated by UV-light and Ha-RAS that binds and phosphorylates the c-Jun activation domain. *Cell* **76** 1025–1037. (doi:10.1016/0092-8674(94)90380-8)
- Dietz JR, Scott DY, Landon CS & Nazian SJ 2001 Evidence supporting a physiological role for proANP (1–30) in the regulation of renal excretion. *American Journal of Physiology* **280** R1510–R1517.
- Doanes AM, Hegland DD, Sethi R, Kovesdi I, Bruder JT & Finkel T 1999 VEGF stimulates MAPK through a pathway that is unique for receptor tyrosine kinases. *Biochemical and Biophysical Research Communications* **255** 545–548. (doi:10.1006/bbrc.1999.0227)
- Duan Z, Foster R, Bell DA, Mahoney J, Wolak K, Vaidya A, Hempel C, Lee H & Seiden MV 2006 Signal transducers and activators of transcription 3 pathway activation in drug-resistant ovarian cancer. *Clinical Cancer Research* **12** 5055–5063. (doi:10.1158/1078-0432.CCR-06-0861)
- Ebert MPA, Yu J, Hoffman J, Rocco A, Rocken C, Kahmann S, Muller O, Korc M, Sung JJ & Malfheiner P 2003 Loss of β -catenin expression in metastatic gastric cancer. *Journal of Clinical Oncology* **2003** 1708–1714. (doi:10.1200/JCO.2003.10.017)
- Eichelbaum EJ, Vesely BA, Alli AA, Sun Y, Gower WR, Jr & Vesely DL 2006 Four cardiac hormones decrease up to 82% of human medullary thyroid carcinoma cells within 24 hours. *Endocrine* **30** 325–332. (doi:10.1007/s12020-006-0011-6)
- Eichelbaum EJ, Sun Y, Alli AA, Gower WR, Jr & Vesely DL 2008 Cardiac hormones and urodilatin eliminate up to 86% of human small-cell lung carcinomas in mice. *European Journal of Clinical Investigation* **38** 562–570. (doi:10.1111/j.1365-2362.2008.01978.x)
- Ferrara N 2004 Vascular endothelial growth factor: Basic science and clinical progress. *Endocrine Reviews* **25** 581–611. (doi:10.1210/er.2003-0027)
- Folkman J 1971 Tumor angiogenesis: therapeutic implications. *New England Journal of Medicine* **295** 1182–1186.
- Folkman J 2007 Angiogenesis: an organizing principle for drug discovery? *Nature Reviews. Drug Discovery* **6** 273–286. (doi:10.1038/nrd2115)
- Franz M, Woloszczuk W & Horl WH 2000 N-terminal fragments of the proatrial natriuretic peptide in patients before and after hemodialysis treatment. *Kidney International* **58** 374–378. (doi:10.1046/j.1523-1755.2000.00175.x)
- Franz M, Woloszczuk W & Horl WH 2001 Plasma concentration and urinary excretion of N-terminal proatrial natriuretic peptides in patients with kidney diseases. *Kidney International* **59** 1928–1934. (doi:10.1046/j.1523-1755.2001.0590051928.x)
- Garcia-Rostan G, Tallini G, Herrero A, D'Aquila TG, Carcangui ML & Rimm DL 1999 Frequent mutation and nuclear localization of β -catenin in anaplastic thyroid carcinoma. *Cancer Research* **59** 1811–1815.
- Gardner DG, Kovacic-Milivojevic BK & Garmai M 1997 Molecular biology of the natriuretic peptides. In *Atrial Natriuretic Peptides*, pp 15–38. Ed DL Vesely. Trivandrum, India: Research Signpost.
- Gerber HP, McMurtrey A, Kowalski J, Yan M, Keyt BA, Dixit V & Ferrara N 1998 Vascular endothelial growth factor regulates endothelial cell survival through the phosphatidylinositol 3'-kinase/AKT signal transduction pathway: requirement for FLK-1/KDR activation. *Journal of Biological Chemistry* **273** 30336–30343. (doi:10.1074/jbc.273.46.30336)
- Geyer FC, Lacroix-Triki M, Savage K, Arnedos M, Lambros MB, MacKay A, Natrajan R & Reis-Filho JS 2010 β -Catenin pathway activation in breast cancer is associated with triple-negative phenotype but not with CTNNB1 mutation. *Modern Pathology* **24** 209–231. (doi:10.1038/modpathol.2010.205)
- Gower WR, Jr, Vesely BA, Alli AA & Vesely DL 2005 Four peptides decrease human colon adenocarcinoma cell number and DNA synthesis via guanosine 3',5'-cyclic monophosphate. *International Journal of Gastrointestinal Cancer* **36** 77–87. (doi:10.1385/IJGC:36:2:77)
- Gower WR, Carter GM, McAfee Q & Solivan SM 2006 Identification, regulation and anti-proliferative role of the NPR-C receptor in gastric epithelial cells. *Molecular and Cellular Biochemistry* **293** 103–118. (doi:10.1007/s11010-006-9234-3)
- Grandis J, Drenning SD, Chakraborty A, Zhou MY, Zeng Q, Pitt AS & Tweardy DJ 1998 Requirement of STAT3 but not STAT1 activation for epidermal growth factor receptor-mediated cell growth *in vitro*. *Journal of Clinical Investigation* **102** 1385–1392. (doi:10.1172/JCI3785)
- Gunning ME, Brady HR, Otuechere G, Brenner BM & Ziedel ML 1992 Atrial natriuretic peptide (31–67) inhibits Na transport in rabbit inner medullary collecting duct cells: role of prostaglandin E_2 . *Journal of Clinical Investigation* **89** 1411–1417. (doi:10.1172/JCI115730)
- Gupta I, Kshirsagar S, Li W, Gui L, Ramakrishnan S, Gupta P, Law PW & Hebbel RP 1999 VEGF prevents apoptosis of human microvascular endothelial cells via opposing effects on MAPK/ERK and SAPK/JNK signaling. *Experimental Cell Research* **247** 495–504. (doi:10.1006/excr.1998.4359)
- Hancock R & Boulikas T 1982 Functional organization in the nucleus. *International Review of Cytology* **79** 165–214.
- Hay N 2005 The AKT–mTOR tango and its relevance to cancer. *Cancer Cell* **8** 179–183. (doi:10.1016/j.ccr.2005.08.008)
- Heasley LE & Han SY 2006 JNK regulation of oncogenesis. *Molecules and Cells* **21** 167–173.
- Heiser PW, Cano DA, Landsman L, Kim GE, Kench JG, Klimstra DS, Taketo MM, Biankin AV & Hebrok M 2008 Stabilization of β -catenin induces pancreas tumor formation. *Gastroenterology* **135** 1288–1300. (doi:10.1053/j.gastro.2008.06.089)

- Hennessy BT, Smith DL, Ram PT, Lu Y & Mills GB 2005 Exploiting the PI3K/AKT pathway for cancer drug discovery. *Nature Reviews. Drug Discovery* **4** 988–1004. (doi:10.1038/nrd1902)
- Hirata H, Hinoda Y, Ueno K, Majid S, Saini S & Dahiya R 2010 Role of secreted frizzled-related protein 3 in human renal cell carcinoma. *Cancer Research* **70** 1896–1905. (doi:10.1158/0008-5472.CAN-09-3549)
- Hoeben A, Landuyt B, Highley MS, Wilders H, Van Oosterom AT & DeBruijn EA 2004 Vascular endothelium growth factor. *Pharmacological Reviews* **56** 549–580. (doi:10.1124/pr.56.4.3)
- Hunter EMF, Kelly PA, Prowse C, Woods FJ & Lowry PJ 1998 Analysis of peptides derived from pro atrial natriuretic peptides that circulate in man and increase in heart disease. *Scandinavian Journal of Clinical and Laboratory Investigation* **58** 205–216. (doi:10.1080/00365519850186599)
- Juneja J, Cushman I & Casey PJ 2011 G12 signaling through c-Jun NH₂-terminal kinase promotes breast cancer cell invasion. *PLoS ONE* **6** e26085. (doi:10.1371/journal.pone.0026085)
- Kamada T & Feramisco JR 1984 Epidermal growth factor stimulates guanine nucleotide-binding activity and phosphorylation of *ns* oncogene proteins. *Nature* **310** 147–150. (doi:10.1038/310147a0)
- Katoh M 2002 Regulation of WNT3 and WNT3A mRNAs in human cancer cell lines NT2, MCF-7, and MKN45. *International Journal of Oncology* **20** 373–377.
- Kawano Y & Kypta R 2003 Secreted antagonists of the WNT signalling pathway. *Journal of Cell Science* **116** 2627–2634. (doi:10.1242/jcs.00623)
- Kraus C, Liehr T, Hulsken J, Behrens J, Birchmeier W, Grzeschik KH & Ballhausen WG 1994 Location of the human β -catenin gene (CTNNB1) to 3p21; a region implicated in tumor development. *Genomics* **23** 272–274. (doi:10.1006/geno.1994.1493)
- Kyriakos JM, Banerjee P, Nikolakaki E, Dai TA, Rubie EA, Ahmad MF, Avruch J & Woodgett JR 1994 The stress-activated protein-kinase subfamily of c-Jun kinases. *Nature* **369** 156–160. (doi:10.1038/369156a0)
- Lainchbury J, Richards AM & Nicholls MG 1997 Brain natriuretic peptide in heart failure. In *Atrial Natriuretic Peptides*, pp 151–158. Ed. DL Vesely. Trivandrum, India: Research Signpost.
- Lane ML, Frost CD, Nguyen JP, Skelton WP, IV, Skelton M & Vesely DL 2012a Potent selective inhibition of STAT3 versus STAT1 by cardiac hormones. *Molecular and Cellular Biochemistry* **371** 209–215. (doi:10.1007/s11010-012-1437-1)
- Lane ML, Santana O, Frost CD, Nguyen J, Guerrero J, Skelton WP, IV, Skelton M & Vesely DL 2012b Cardiac hormones are c-Jun-N-terminal kinase 2-inhibiting peptides. *Anticancer Research* **32** 721–726.
- Lenz A, Sun Y, Eichelbaum EJ, Skelton WP, IV, Pi G & Vesely DL 2010 Twice weekly intravenous treatment of pancreatic cancer with atrial natriuretic peptide and vessel dilator. *In Vivo* **24** 125–130.
- Li J, Mizukami Y, Zhang X, Jo W-S & Chung DC 2005 Oncogenic KRAS stimulates WNT signaling in colon cancer through inhibition of GSK-3 β . *Gastroenterology* **128** 1907–1918. (doi:10.1053/j.gastro.2005.02.067)
- Lin K, Wang S, Julius MA, Kitajewski J, Moos M, Jr & Luyten FP 1997 The cysteine-rich frizzled domain of Frzb-1 is required and sufficient for modulation of WNT signaling. *PNAS* **94** 11196–11200. (doi:10.1073/pnas.94.21.11196)
- Lin SY, Xia W, Wang JC, Kwong KY, Spohn B, Wen Y, Pestell RG & Hung MC 2000 β -Catenin, a novel prognostic marker for breast cancer: its roles in cyclin D1 expression and cancer progression. *PNAS* **97** 4262–4266. (doi:10.1073/pnas.060025397)
- Liotta LA, Kleinerman J & Saidel GM 1974 Quantitative relationships of intravascular tumor cells, tumor vessels, and pulmonary metastases following tumor implantation. *Cancer Research* **34** 997–1004.
- Lowe AW, Olsen M, Hao Y, Lee SP, Lee KT, Chen X, Vanderijn M & Brown PO 2003 Gene expression patterns in pancreatic tumor, cells and tissues. *Annals of Surgical Oncology* **10** 284–290. (doi:10.1245/ASO.2003.05.003)
- Maiti S, Alam R, Amos CI & Huff V 2000 Frequent association of β -catenin and WTI mutations in Wilms tumors. *Cancer Research* **60** 6288–6292.
- Malbon CC 2004 Frizzleds: new members of the superfamily of G-protein-coupled receptors. *Frontiers in Bioscience* **9** 1048–1058. (doi:10.2741/1308)
- Mann B, Gelos M, Siedow A, Hanski ML, Gratchev A, Ilyas M, Bodmer WF, Moyer MP, Riecken EO, Burh HJ *et al.* 1999 Target genes of β -catenin-T cell factor/lymphoid-enhancer-factor signaling in human colorectal carcinomas. *PNAS* **96** 1603–1608. (doi:10.1073/pnas.96.4.1603)
- Martin DR, Pevanouse JB, Trigg DJ, Vesely DL & Buerkert JE 1990 Three peptides from the ANF prohormone NH₂-terminus are natriuretic and/or kaliuretic. *American Journal of Physiology* **258** F1401–F1408.
- McCubrey JA, Steelman LS, Chappell WH, Abrams SL, Wong EWT, Chang F, Lehmann B, Terrian DM, Milella M, Tafuri A *et al.* 2007 Roles of the RAF/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. *Biochimica et Biophysica Acta* **1773** 1263–1284. (doi:10.1016/j.bbamcr.2006.10.001)
- McCubrey JA, Milella M, Tafuri A, Martelli AM, Lunghi P, Bonati A, Cervello M, Lee JT & Steelman LS 2008 Targeting the RAF/MEK/ERK pathway with small-molecule inhibitors. *Current Opinion in Investigational Drugs* **9** 614–633.
- McMahon G 2000 VEGF receptor signaling in tumor angiogenesis. *Oncologist* **5** S3–S10. (doi:10.1634/theoncologist.5-suppl_1-3)
- Meadows KN, Bryant P & Pumiglia K 2001 Vascular endothelial growth factor induction of the angiogenic phenotype required RAS activation. *Journal of Biological Chemistry* **276** 49289–49298. (doi:10.1074/jbc.M108069200)
- Medema RS, DeVries-Smith AMM, Van der Zon GCM, Maassen JA & Bos JL 1992 Ras activation by insulin and epidermal growth factor through enhanced exchange of guanine nucleotides on p²¹ ras. *Molecular and Cellular Biology* **13** 155–162.
- Minden A, Lin A, McMahon M, Lange-Carter C, Derijard B, Davis RJ, Johnson GL & Karin M 1994 Differential activation of ERK and JNK mitogen-activated protein kinases by RAF-1 and MEKK. *Science* **266** 1719–1723. (doi:10.1126/science.7992057)
- Mirabelli-Primidahl L, Gryfe R, Kim H, Millar A, Luceri C, Dale D, Holowaty E, Bayat B, Gallinger S & Redston M 1999 β -Catenin mutations are specific for colorectal carcinomas with microsatellite instability but occur in endometrial carcinomas irrespective of mutator pathway. *Cancer Research* **59** 3346–3351.
- Morin PJ 1999 β -Catenin signaling and cancer. *BioEssays* **21** 1021–1030. (doi:10.1002/(SICI)1521-1878(199912)22:1<1021::AID-BIES6>3.0.CO;2-P)
- Nguyen JP, Frost CD, Lane ML, Skelton WP, IV, Skelton M & Vesely DL 2012 Novel dual inhibitors of vascular endothelial growth factor and VEGFR2 receptor. *European Journal of Clinical Investigation* **42** 1061–1067. (doi:10.1111/j.1365-2362.2012.02695.x)
- Niles AL, Moravec RA, Hesselberth E, Scurria MA, Daily WJ & Riss TL 2007 A homogenous assay to measure live and dead cells in the same sample by detecting different protease markers. *Analytical Biochemistry* **366** 197–206. (doi:10.1016/j.ab.2007.04.007)
- Okada R, Rak JW, Croix BS, Lieubeau B, Kaya M, Roncari L, Shirasawa S, Sasazuki T & Kerbel RS 1998 Impact of oncogenes in tumor angiogenesis: mutant KRAS up-regulation of vascular endothelial growth factor/vascular permeability factor is necessary, but not sufficient for tumorigenicity of human colorectal carcinoma cells. *PNAS* **95** 3609–3614. (doi:10.1073/pnas.95.7.3609)
- Pi GE, Skelton WP, IV & Vesely DL 2011 Four cardiac hormones cause cytotoxicity of human cancer cells but not of healthy cells. *Cancer Therapy* **8** 44–50.
- Pitchumoni CS 1998 Pancreatic disease. In *Internal Medicine*, 5th edn, pp 2233–2247. Ed JH Stein. St Louis: Mosby.
- Polakis P 2012 Drugging WNT signaling in cancer. *EMBO Journal* **31** 2737–2746. (doi:10.1038/emboj.2012.126)
- Qui MS & Green ST 1991 NGF and BGF rapidly activate p21 ras in PC12 cells by distinct, convergent pathways involving tyrosine phosphorylation. *Neuron* **7** 937–946. (doi:10.1016/0896-6273(91)90339-2)

- Rashed HM, Su H & Patel TB 1993 Atrial natriuretic peptide inhibits growth of hepatoblastoma (HEP G2) cells by means of activation of clearance receptors. *Hepatology* **17** 677–684. (doi:10.1002/hep.1840170423)
- Rattner A, Hsieh JC, Smallwood PM, Gilbert DJ, Copeland NG, Jenkins NA & Nathans J 1997 A family of secreted proteins contains homology to the cysteine-rich ligand-binding domain of frizzled receptors. *PNAS* **94** 2859–2863. (doi:10.1073/pnas.94.7.2859)
- Rosette C & Karin M 1996 Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. *Science* **274** 1194–1197. (doi:10.1126/science.274.5290.1194)
- Roy HK, Olusola BF, Clemens DL, Karolski WJ, Ratashak A, Lynch HT & Smyrk TC 2002 AKT proto-oncogene overexpression is an early event during sporadic colon carcinogenesis. *Carcinogenesis* **23** 201–205. (doi:10.1093/carcin/23.1.201)
- Rubin JS, Barshishat-Kupper M, Feroze-Merzoup F & Xi ZF 2006 Secreted WNT antagonists are tumor suppressors: pro and con. *Frontiers in Bioscience* **11** 2093–2105. (doi:10.2741/1952)
- Satoh T, Endo M, Nakaruku M, Akiyana T, Yamamoto T & Kazio Y 1990 Accumulation of p21 ras GTP in response to stimulation with epidermal growth factor and oncogene products with tyrosine activity. *PNAS* **87** 7926–7929. (doi:10.1073/pnas.87.20.7926)
- Schindler C, Shuai K, Prezioso VR & Darnell JE, Jr 1992 Interferon-dependent tyrosine phosphorylation of a latent cytoplasmic transcription factor. *Science* **257** 809–813. (doi:10.1126/science.1496401)
- Schlessinger J 2000 Cell signaling by receptor tyrosine kinases. *Cell* **103** 211–225. (doi:10.1016/S0092-8674(00)00114-8)
- Scholl FA, Dumesic PA & Khavari PA 2005 Effects of active MEK 1 expression *in vivo*. *Cancer Letters* **230** 1–5. (doi:10.1016/j.canlet.2004.12.013)
- Scotland RS, Ahluwalia A & Jobbs AJ 2005 C-type natriuretic peptide in vascular physiology and disease. *Pharmacology & Therapeutics* **105** 85–93. (doi:10.1016/j.pharmthera.2004.08.011)
- Sebolt-Leopold JS 2008 Advances in the development of cancer therapeutics directed against the RAS mitogen-activated protein kinase pathway. *Clinical Cancer Research* **14** 3651–3656. (doi:10.1158/1078-0432.CCR-08-0333)
- Serafino A, Moroni N, Psaila R, Zonfrillo M, Andreola F, Wannenes F, Mercuri L, Rasi G & Pierimarc P 2012 Anti-proliferative effect of atrial natriuretic peptide on colorectal cancer cells: evidence for an Akt-mediated cross-talk between NHE-1 activity and Wnt/ β -catenin signaling. *Biochimica et Biophysica Acta* **1822** 1004–1018. (doi:10.1016/j.bbdis.2012.02.016)
- Shaw RJ & Cantley LC 2006 Ras, PI(3)K and mTOR signaling controls tumor cell growth. *Nature* **441** 424–430. (doi:10.1038/nature04869)
- Skelton WP, IV, Pi GE & Vesely DL 2012 Four cardiac hormones cause death of human cancer cells but not of healthy cells. *Anticancer Research* **31** 395–402.
- Skelton WP, IV, Skelton M & Vesely DL 2013a Cardiac hormones are potent inhibitors of secreted frizzled related protein-3 in human cancer cells. *Experimental and Therapeutic Medicine* **5** 475–478.
- Skelton WP, IV, Skelton M & Vesely DL 2013b Inhibition of AKT in human pancreatic, renal and colorectal cancer cells by four cardiac hormones. *Anticancer Research* **33** 785–790.
- Skelton WP, IV, Skelton M & Vesely DL 2013 Cardiac hormones are novel inhibitors of Wnt-3a in human cancer cells. *Cancer Therapy* **9** 24–29.
- Song L, Turkson J, Karras JG, Jove R & Haura EB 2003 Activation of STAT3 by receptor tyrosine kinases and cytokines regulates survival in human non-small-cell carcinoma cells. *Oncogene* **22** 4150–4165. (doi:10.1038/sj.onc.1206479)
- Staal SP, Hartley JW & Rowe SP 2007 Isolation of transforming murine leukemia viruses from mice with a high incidence of spontaneous lymphoma. *PNAS* **74** 3065–3067. (doi:10.1073/pnas.74.7.3065)
- Sun Y, Eichelbaum EJ, Wang H & Vesely DL 2006a Vessel dilator and kaliuretic peptide inhibit activation of ERK 1/2 in human prostate cancer cells. *Anticancer Research* **26** 3217–3222.
- Sun Y, Eichelbaum EJ, Wang H & Vesely DL 2006b Atrial natriuretic peptide and long acting natriuretic peptide inhibit ERK 1/2 in prostate cancer cells. *Anticancer Research* **26** 4143–4148.
- Sun Y, Eichelbaum EJ, Wang H & Vesely DL 2007a Vessel dilator and kaliuretic peptide inhibit MEK 1/2 activation in human prostate cancer cells. *Anticancer Research* **27** 1387–1392.
- Sun Y, Eichelbaum EJ, Wang H & Vesely DL 2007b Atrial natriuretic peptide and long acting natriuretic peptide inhibit MEK 1/2 activation in human prostate cancer cells. *Anticancer Research* **27** 3813–3818.
- Sun Y, Eichelbaum EJ, Wang H & Vesely DL 2007c Insulin and epidermal growth factor activation of ERK 1/2 and DNA synthesis is inhibited by four cardiac hormones. *Journal of Cancer Molecules* **3** 113–120.
- Sun Y, Eichelbaum EJ, Skelton WP, IV, Lenz A, Regales N, Wang H & Vesely DL 2009a Vessel dilator and kaliuretic peptide inhibit RAS in human prostate cancer cells. *Anticancer Research* **29** 971–975.
- Sun Y, Eichelbaum EJ, Lenz A, Skelton WP, IV, Wang H & Vesely DL 2009b Atrial natriuretic peptide and long-acting natriuretic peptide inhibit RAS in human prostate cancer cells. *Anticancer Research* **29** 1889–1893.
- Sun Y, Eichelbaum EJ, Lenz A, Wang H & Vesely DL 2009c Four cardiac hormones inhibit insulin's mitogenic action via inhibiting RAS. *Cancer Therapy* **7** 367–372.
- 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. (doi:10.1111/j.1365-2362.2010.02283.x)
- Thompson MD & Monga SPS 2007 WNT/ β -catenin signaling in liver health and disease. *Hepatology* **45** 1298–1305. (doi:10.1002/hep.21651)
- Vesely DL 1977 Signal transduction: activation of guanylate cyclase-cyclic guanosine-3',5' monophosphate system by hormones and free radicals. *American Journal of the Medical Sciences* **314** 311–323. (doi:10.1097/0000441-199711000-00008)
- Vesely DL 1992 *Atrial Natriuretic Hormones*, pp 1–256. Englewood Cliffs, New Jersey: Prentice Hall.
- Vesely DL 2002 Atrial natriuretic peptide prohormone gene expression: hormones and diseases that upregulate its expression. *IUBMB Life* **53** 153–159. (doi:10.1080/15216540212336)
- Vesely DL 2003 Natriuretic peptides and acute renal failure (review). *American Journal of Physiology* **285** F167–F177.
- Vesely DL, Norris JS, Walters JM, Jespersen RR & Baeyens DA 1987 Atrial natriuretic prohormone peptides 1–30, 31–67 and 79–98 vasodilate the aorta. *Biochemical and Biophysical Research Communications* **148** 1540–1548. (doi:10.1016/S0006-291X(87)80307-8)
- Vesely DL, Norsk P, Winters CJ, Rico DM, Sallman AL & Epstein M 1989 Increased release of the N-terminal and C-terminal portions of the prohormone of atrial natriuretic factor during immersion-induced central hypervolemia in normal humans. *Proceedings of the Society for Experimental Biology and Medicine* **192** 230–235.
- Vesely DL, Douglass MA, Dietz JR, Gower WR, Jr, McCormick MT, Rodriguez-Paz G & Schocken DD 1994a Three peptides from the atrial natriuretic factor prohormone amino terminus lower blood pressure and produce diuresis, natriuresis, and/or kaliuresis in humans. *Circulation* **90** 1129–1140. (doi:10.1161/01.CIR.90.3.1129)
- Vesely DL, Douglass MA, Dietz JR, Giordano AT, McCormick MT, Rodriguez-Paz G & Schocken DD 1994b Negative feedback of atrial natriuretic peptides. *Journal of Clinical Endocrinology and Metabolism* **78** 1128–1134. (doi:10.1210/jc.78.5.1128)
- Vesely DL, Dietz JR, Parks JR, Baig M, McCormick MT, Cintron G & Schocken DD 1998 Vessel dilator enhances sodium and water excretion and has beneficial hemodynamic effects in persons with congestive heart failure. *Circulation* **98** 323–329. (doi:10.1161/01.CIR.98.4.323)
- Vesely BA, McAfee Q, Gower WR, Jr & Vesely DL 2003 Four peptides decrease the number of human pancreatic adenocarcinoma cells. *European Journal of Clinical Investigation* **33** 998–1005. (doi:10.1046/j.1365-2362.2003.01262.x)
- Vesely DL, Clark LC, Garces AH, McAfee QW, Soto J & Gower WR, Jr 2004 Novel therapeutic approach for cancer using four cardiovascular

- hormones. *European Journal of Clinical Investigation* **34** 674–682. (doi:10.1111/j.1365-2362.2004.01402.x)
- Vesely BA, Alli AA, Song S, Gower WR, Jr, Sanchez-Ramos J & Vesely DL 2005a Four peptide hormones specific decrease (up to 97%) of human prostate carcinoma cells. *European Journal of Clinical Investigation* **35** 700–710. (doi:10.1111/j.1365-2362.2005.01569.x)
- Vesely BA, Song S, Sanchez-Ramos J, Fitz SR, Alli A, Solivan SM, Gower WR, Jr & Vesely DL 2005b Five cardiac hormones decrease the number of human small-cell lung cancer cells. *European Journal of Clinical Investigation* **35** 388–398. (doi:10.1111/j.1365-2362.2005.01501.x)
- Vesely BA, Song S, Sanchez-Ramos J, Fitz SR, Solivan SR, Gower WR, Jr & Vesely DL 2005c Four peptide hormones decrease the number of human breast adenocarcinoma cells. *European Journal of Clinical Investigation* **35** 60–69. (doi:10.1111/j.1365-2362.2005.01444.x)
- Vesely BA, Alli A, Song S, Sanchez-Ramos J, Fitz SR, Gower WR, Jr & Vesely DL 2006a Primary malignant tumors of the heart: four cardiovascular hormones decrease the number and DNA synthesis of human angiosarcoma cells. *Cardiology* **105** 226–233. (doi:10.1159/000091816)
- Vesely BA, Fitz SR, Gower WR, Jr & Vesely DL 2006b Vessel dilator: most potent of the atrial natriuretic peptides in decreasing the number and DNA synthesis of human squamous lung cancer cells. *Cancer Letters* **233** 226–231. (doi:10.1016/j.canlet.2005.03.024)
- Vesely BA, Eichelbaum EJ, Alli AA, Sun Y, Gower WR, Jr & Vesely DL 2006c Urodilatin and four cardiac hormones decrease human renal carcinoma cell number. *European Journal of Clinical Investigation* **36** 810–819. (doi:10.1111/j.1365-2362.2006.01721.x)
- Vesely BA, Eichelbaum EJ, Alli AA, Sun Y, Gower WR, Jr & Vesely DL 2007a Four cardiac hormones eliminate 4-fold more human glioblastoma cells than green mamba snake peptide. *Cancer Letters* **254** 94–101. (doi:10.1016/j.canlet.2007.02.015)
- Vesely BA, Eichelbaum EJ, Alli AA, Sun Y, Gower WR, Jr & Vesely DL 2007b Four cardiac hormones cause cell death in 81% of human ovarian adenocarcinoma cells. *Cancer Therapy* **5** 97–104.
- Vesely DL, Eichelbaum EJ, Sun Y, Alli AA, Vesely BA, Luther SL & Gower WR, Jr 2007c Elimination of up to 80% of human pancreatic adenocarcinomas in athymic mice by cardiac hormones. *In Vivo* **21** 445–452.
- Vesely DL, Vesely BA, Eichelbaum EJ, Sun Y, Alli AA & Gower WR, Jr 2007d Four cardiac hormones eliminate up to two-thirds of human breast cancers in athymic mice. *In Vivo* **21** 973–978.
- Villarreal D, Reams GP, Taraben A & Freeman RH 1999 Hemodynamic and renal effects of proANF 31–67 in hypertensive rats. *Proceedings of the Society for Experimental Biology and Medicine* **221** 166–170. (doi:10.1046/j.1525-1373.1999.d01-71.x)
- Vivanco I & Sawyers CL 2002 The phosphatidylinositol 3-kinase–AKT pathway in human cancer. *Nature Reviews. Cancer* **2** 489–501. (doi:10.1038/nrc839)
- Voeller JH, Trucia CI & Gelmann EP 1998 β -Catenin mutations in human prostate cancer. *Cancer Research* **58** 2520–2523.
- Waldman SE, Rapoport RM & Murad F 1984 Atrial natriuretic factor selectively activates membranous guanylate cyclase and elevates cyclic GMP in rat tissues. *Journal of Biological Chemistry* **259** 14332–14334.
- Wang X, Goode EL, Fredericksen ZA, Vierkant RA, Pankratz VS, Liu-Mares W, Rider DN, Vachon CM, Cerhan JR, Olson JE *et al.* 2008 Association of genetic variation in genes implicated in the β -catenin destruction complex with risk of breast cancer. *Cancer Epidemiology, Biomarkers, and Prevention* **17** 2101–2108. (doi:10.1158/1055-9965.EPI-08-0134)
- Wang K, Jiang YZ, Chen DB & Zheng J 2009 Hypoxia enhances FGF-2 and VEGF stimulated human placental artery endothelial cell proliferation: roles of MEK-1/2/ERK1-2 and PI3K/AKT1 pathways. *Placenta* **30** 1045–1051. (doi:10.1016/j.placenta.2009.10.007)
- Wang X, Raulji P, Mohapatra SS, Patel R, Hellermann G, Kong X, Vera PD, Meyer-Siegler KL, Coppola D & Mohapatra S 2011 Natriuretic peptide receptor A as a novel target for prostate cancer. *Molecular Cancer* **10** 56. (doi:10.1186/1476-4598-10-56)
- Winters CJ, Sallman AL, Baker BJ, Meadows J, Rico DM & Vesely DL 1989 The N-terminus and a 4000 molecular weight peptide from the mid portion of the N-terminus of the atrial natriuretic factor prohormone each circulate in humans and increase in congestive heart failure. *Circulation* **80** 438–449. (doi:10.1161/01.CIR.80.3.438)
- Wolf RA, Crane CH, Li D & Evans DB 2010 Neoplasms of the exocrine pancreas. In *Cancer Medicine*, 8th edn, pp 1144–1171. Eds WK Hong, RC Bast Jr, WN Hait, DW Kufe, RE Pollock, RR Weichselbaum, JF Holland & E Frei III. London: BC Decker, Inc.
- Wu J, Harrison JK, Dent P, Lynch KR, Weber MJ & Sturgill TW 1993 Identification and characterization of a new mammalian mitogen-activated protein kinase kinase MKK-2. *Molecular and Cellular Biology* **13** 4539–4548.
- Xia L, Dickens M, Raingeaud J, David RJ & Greenberg ME 1995 Opposing effects of ERK and JNK–p38 MAP kinases on apoptosis. *Science* **270** 1326–1331. (doi:10.1126/science.270.5240.1326)
- Xu YK & Nusse R 1998 The frizzled CRD domain is conserved in diverse proteins including several receptor tyrosine kinases. *Current Biology* **8** R405–R406. (doi:10.1016/S0960-9822(98)70262-3)
- Yang YM, Bost F, Charbono W, Dean N, McKay R, Rhim JS, Depatie C & Mercola D 2003 c-Jun NH(2)-terminal kinase mediates proliferation and tumor growth of human prostate carcinoma. *Clinical Cancer Research* **9** 391–401.
- Yu H & Jove R 2004 The STATs of cancer: new molecular targets come of age. *Nature Reviews. Cancer* **4** 450–456. (doi:10.1038/nrc1275)
- Zeidel ML 1995 Regulation of collecting duct Na^+ reabsorption by ANP 31–67. *Clinical and Experimental Pharmacology & Physiology* **22** 121–124. (doi:10.1111/j.1440-1681.1995.tb01967.x)
- Zeitlin S, Parent A, Silverstein S & Efstratiadis J 1987 Pre mRNA splicing and the nuclear matrix. *Molecular and Cellular Biology* **7** 111–120.
- Zhang X, Gaspard JP & Chung DC 2001 Regulation of vascular endothelial growth factor by the WNT and KRAS pathways in colonic neoplasia. *Cancer Research* **61** 6050–6054.

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