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Review

Family of Peptides Synthesized in the Human Body Have Anticancer Effects

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Abstract. *Four peptides synthesized in the heart, namely atrial natriuretic peptide (ANP), vessel dilator, kaliuretic peptide and long-acting natriuretic peptide (LANP), reduce cancer cells in vitro by up to 97%. These four cardiac hormones, in vivo, eliminate up to 86% of human small-cell lung carcinomas, two-thirds of human breast carcinomas, 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 guanosine triphosphate (RAS) (95% inhibition)-mitogen activated protein kinase kinase 1/2 (MEK-1/2) (98% inhibition)-extracellular signal-related kinases 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 their downstream targets of VEGF is β-Catenin, which they reduce up to 88%. The Wingless-related integration site (WNT) pathway is inhibited by up to 68% and WNT secreted-Frizzled related protein-3 was reduced by up to 84% by the four peptide hormones. A serine/threonine-protein kinase, AKT, derived from "AK" mouse strain with thymomas (T), is reduced by up to 64% by the peptide hormones. Signal transducer and activator of transcription 3 (STAT3), a final "switch" that activates gene expression patterns that lead to malignancy, is decreased by up to 88% by these peptide hormones; STAT3 is specifically reduced as they do not affect STAT1. There is 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 peptides. These peptides have been demonstrated to enter the nucleus of cancer cells where they inhibit the proto-oncogenes c-FOS (up to 82%) and c-JUN (up to 61%). Conclusion: The cardiac peptides inhibit multiple targets and cross-talk between the targets within cancer cells.*

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The human body synthesizes a number of peptides that have salt-excreting (natriuretic) properties to help control blood volume by causing a natriuresis and diuresis in healthy humans (1-3) and in persons retaining salt and water such as in congestive heart failure (4, 5) and acute renal failure (6, 7). In the heart, the atrial natriuretic peptide prohormone (proANP) gene encodes a 126-amino-acid (a.a.) pro-hormone which contains four peptide hormones (3, 8, 9). These four hormones synthesized by the atrium of the heart are long-acting natriuretic peptide (LANP), which consists of the first 30 a.a. from the N-terminal end of the 126 a.a. prohormone, vessel dilator, a.a. 31-67 of this prohormone, kaliuretic peptide, a.a. 79-98; and atrial natriuretic peptide (ANP), a.a. 99-126 of this 126-a.a. pro-hormone (3, 10). These peptides were named for their most potent known biological effect(s) at the time of naming (3). These peptide hormones are now synthesized with commercial peptide synthesizers from their known a.a. sequences (3). *Via* a separate gene, the heart also synthesizes brain natriuretic peptide (BNP), which was misnamed, as 50-fold more BNP is made in the heart than the brain. A third gene in the heart synthesizes C-natriuretic peptide.

In the kidney, the ANP pro-hormone is also synthesized but it is cleaved differently, adding four a.a. of kaliuretic

peptide to the a.a. of ANP (11-13), with the resulting peptide being named urodilatin. It is important to note that the a.a. in urodilatin are identical to those of ANP and the four C-terminal a.a. of kaliuretic peptide (11-13). Thus, one would expect that urodilatin and ANP would have similar if not identical effects, and in general they do (11, 12).

There is also another peptide (DNP) with similar structure to ANP that is found in the venom of the green mamba snake, *Dendroaspis angusticeps* (14). Since DNP has a similar structure to ANP, one would expect that it would have similar effects to ANP (14). Each of these peptides, except BNP, have anticancer effects *in vitro* when given in concentrations above those normally circulating in the human body, *i.e.* pharmacological concentrations (15-26). This review concentrates on their anticancer effects.

It has been reviewed previously (27) that in cell culture, the four cardiac hormones reduce up to 97% of human pancreatic, colon, prostate, breast, ovarian and kidney adenocarcinoma cells (15-21), angiosarcoma of the heart cells (22), melanomas (23), medullary thyroid carcinomas (21), glioblastomas of the brain (24) as well as small-cell (25) and squamous cell lung carcinoma cells (26). The heart and kidney peptides eliminate up to 80% of human pancreatic carcinomas (28), 86% of small cell lung carcinomas (29) and two-thirds of human breast carcinomas growing in athymic mice (30), which was detailed in the previous review (27), as was part of their mechanism(s) of action illustrated in Figure 1. The previous review detailed that these peptides cause up to 95% inhibition of the conversion of inactive RAS-GDP to active RAS-GTP (31, 32), up to 98% inhibition of MEK-1/2 kinases (33, 34) and up to 96% of ERK-1/2 kinases (35, 36) in the RAS–MEK-1/2–ERK-1/2 kinase cascade and that they also inhibit up to 89% of c-JUN-N-terminal kinase 2 (JNK2) (37) whose activation is dependent upon RAS (38, 39).

The present review concentrates on the new information gained since the previous review (27) focused on the mechanism(s) of action of these anticancer agents.

WNT Signaling Pathway

The WNT signaling pathway is a signal transduction pathway that is enhanced in a variety of cancer types (40, 41). 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 (41). WNT signaling is stimulated by RAS (42) and vascular endothelial growth factor (VEGF) pathways (42). Both RAS and VEGF contribute to the pathobiology of colon cancer, in part through the WNT pathway (43). The fou-peptide hormones from the heart maximally reduce WNT3α 68% in human pancreatic carcinoma cells (44).

Vascular Endothelial Growth Factor

VEGF plays an essential role throughout tumor development by enabling blood vessels to establish and grow into tumors, thereby providing nutrients and oxygen to the tumor (45-49). VEGF intracellularly enables cancer cells to grow *via* stimulating RAS (50, 51), MEK-1/2 (52, 53) and ERK-1/2 kinases (54, 55). VEGFR2/KDR/FLK-1 receptor is the main VEGF receptor mediating the cancer-enhancing effects of VEGF (46, 48, 56).

The four cardiac peptides from the *ANP* pro-hormone gene reduce the VEGFR2 receptor in human pancreatic adenocarcinoma cells by up to 83% (57). They also reduce the VEGFR2 by up to 89% in human small-cell lung cancer cells and up to 92% in human prostate cancer cells (57). These results were confirmed by western blottting (57). The cardiac hormones reduce VEGF itself by up to 58% (57). Although there are a number of compounds that inhibit VEGF or its receptor, VEGFR2, the cardiac peptides are the first agents that are dual inhibitors of VEGF and VEGFR2 (57).

β-Catenin

One of the downstream targets of VEGF is β-Catenin (58). β-Catenin is a multi-functional protein located at the intracellular side of the cytoplasmic membrane that causes the malignant growth of pancreatic (59, 60), colonic (40, 61) and renal (62, 63) tumors. β-Catenin activation also leads to gastric (64), breast (65, 66), liver (67), ovarian (68), endometrial (68), anaplastic thyroid (69, 70), and prostate (71, 72) cancer.

The four cardiac peptide hormones reduce 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 (73). 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 (74). ANP causes a down-regulation of *c-Myc* (*MYC*) and cyclin D-1 gene transcription regulated by β-Catenin (74). β-catenin appears to be the central target of the anticancer effects of these cardiac hormones since these hormones inhibit upstream RAS kinase, which activates β-Catenin (70), and downstream JNK and VEGF, which are activated by β-Catenin, as illustrated in Figure 1 (58, 75).

AKT

AKT, also known as protein kinase B, is a serine/threonine protein kinase that has a key role in cell proliferation and in the growth of many types of cancer (76-80). The name AKT derives from the 'Ak' mouse strain that develops spontaneous thymic lymphomas, and 'T' stands for thymoma (81). AKT is overexpressed in colorectal cancer cells but not in normal

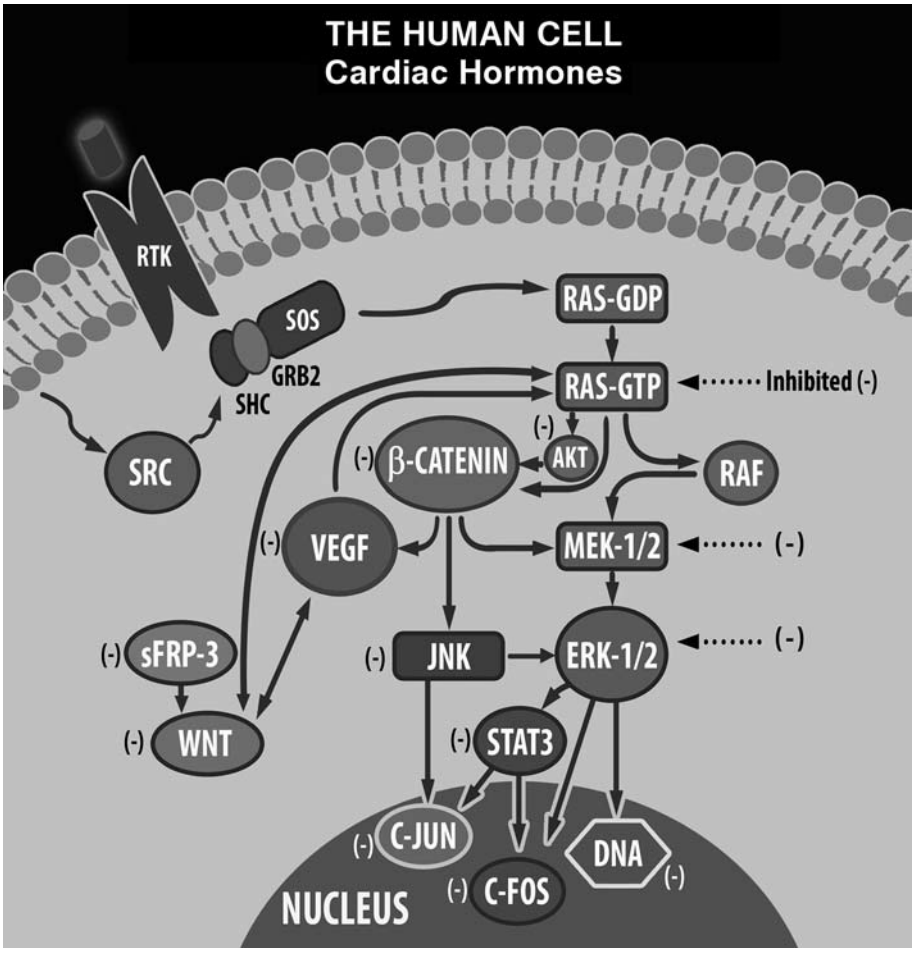

Figure 1. *Cardiac hormones inhibit cellular oncogenes c-FOS (up to 82%) and c-JUN (up to 65%) and rat sarcoma-bound guanosine GTP (RAS-GTP), mitogen-activated protein kinase kinase 1/2 (MEK-1/2), and extracellular signal-related kinases 1/2 (ERK-1/2) kinase cascade by 95-98%. These multiple kinase inhibitors are also strong inhibitors (i.e. by 91%) of DNA synthesis within cancer cells. Other targets which the cardiac hormones inhibit within cancer cells are vascular endothelial growth factor (VEGF), the VEGFR2 receptor, β-Catenin, secreted frizzled-related protein 3 (sFRP-3), c-JUN-N-terminal kinase 2 (JNK), signal transducer and activator of transcription 3 (STAT3) and the WNT pathway. As illustrated, cardiac hormones inhibit [shown by (–)] several steps in the feedback loop that stimulate the oncogenes c-FOS and c-JUN in the nucleus, interrupting the vicious cycle of stimulating cancer cell growth. 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 guanosine diphosphate (GDP); RAG: rapidly accelerated fibrosarcoma serine/threonine protein kinase; AKT: AK mouse strain with 'T' for thymoma. Modified with permission from Reference 120.*

colonic mucosa or hyperplastic polyps (82). ANP reduces the activation of AKT by two-fold between 2 and 4 h of treatment in cell culture (74). Vessel dilator, kaliuretic peptide, and LANP reduce the concentration of AKT by 60%, 61% and 59% in human pancreatic carcinoma cells, by 47%, 45%, and 46% in human colorectal cancer cells, and by 31%, 32%, and 31% in renal adenocarcinoma cells (83). There is cross-talk between the activation of AKT and its inhibition by the cardiac peptides, which is summarized as follows: RAS activates AKT (84). Growth factors such as epidermal growth factor also activate RAS, with a resultant downstream activation of AKT (84). The effects of VEGFs on cancer growth and metastasis are mediated by binding the VEGFR2 (KDR/FLK-1) receptor, which, in turn, activates the AKT pathway (85). The four cardiac peptides inhibit each of these steps. Thus, there is a complex interplay of AKT, RAS, and VEGF in causing cancer and maintaining cancer cell growth (58, 76, 77, 85, 86). This interplay is modified (inhibited) by these four cardiac peptides.

Secreted Frizzled-related Protein-3

Secreted frizzled-related protein-3 (sFRP-3), a ~300-a.a. glycoprotein (87-90), promotes renal cancer growth when injected into athymic mice (91). sFRP-3 also causes tumor

promotion in other types of cancers (92). ANP affects activation of the frizzled-receptor (74) which contains sFRP-3 (93, 94). ANP and the frizzled receptor co-localize on the cell membrane within 30 min after ANP addition to culture medium (74). Vessel dilator, kaliuretic peptide, ANP and LANP reduce 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 (95). With respect to the mechanism by which the reduction of sFRP-3 levels by the cardiac peptides leads to their anticancer effects, their ability to inhibit sFRP-3, the active cysteine-rich domain (CRD) of the frizzled receptor (88), blocks the propagation of the signal responsible for causing cancer cell growth.

Signal Transducers and Activators of Transcription

STATs are cytoplasmic transcription factors (Figure 1) (96, 97) which are the final 'switches' that activate gene expression patterns that lead to cancer (96-98). STAT3 is important in human cancer formation (97, 99). STAT3 is overexpressed in a variety of human tumors (97, 100, 101). The epidermal growth factor (EGF) receptor-mediated growth of squamous carcinoma cells is known to require STAT3 but not STAT1 (100).

ERK-1/2 activates (*i.e.* phosphorylates) STAT3 at serine 727 in response to growth factors (102). STAT3 is an excellent substrate for ERK kinases (102) and, as above, the cardiac peptides all inhibit ERK-1/2 kinases. Vessel dilator, LANP, kaliuretic peptide, and ANP reduce STAT3 by 88%, 54%, 55%, and 65% respectively in human small-cell lung cancer cells, and by 66%, 57%, 70%, and 77% in human pancreatic adenocarcinoma cells (103). These peptides from the heart do not reduce STAT1 in either human small-cell lung cancer or pancreatic adenocarcinoma cells (103). Thus, the four cardiac peptides are significant inhibitors of STAT3 but spare STAT1, which suggests a specificity for the anticancer mechanism(s) of action of these peptides in human cancer cells (103).

Oncogenes

c-FOS is a cellular proto-oncogene belonging to the immediate early gene family of transcription factors (104, 105). Transcription of *c-FOS* is up-regulated in response to growth factors such as EGF (104, 106). *c-FOS* overexpression increases proliferation of human hepatocytes (107), and enhanced *c-FOS* expression helps induce hepatocellular carcinomas (108-110). *c-FOS* dimerizes with c-JUN to form activator protein 1 (AP-1) transcription factor, which up-regulates transcription of genes involved in proliferation and cancer formation (105, 111). When *c-FOS* and *c-JUN* are joined to form AP-1 protein, this protein can bind to the AP-1-binding site on DNA to induce transcription of various genes (112). The AP-1 complex has been associated with transformation and progression of cancer (105). Regulation of *c-FOS* is performed through the MAPK pathway and *via* STAT3 (113, 114) (Figure 1). *c-JUN* is another proto-oncogene which is activated through double phosphorylation by the JNK pathway and STAT3 (114-116) (Figure 1). Amongst the *JUN* proteins, *c-JUN* is unique in positively-regulating cell proliferation (105).

Vessel dilator, LANP, kaliuretic peptide, and ANP have each been demonstrated by immunocytochemical techniques to enter the nucleus of cancer cells (117, 118) where they inhibited proto-oncogenes. Indeed, this is the case, as demonstrated in three different cancer lines (119). Thus, vessel dilator, LANP, kaliuretic peptide and ANP over a concentration range of 100 pM-10 μM, reduce *c-FOS* by 61%, 60%, 61% and 59% in human hepatocellular cancer cells, by 82%, 74%, 78% and 74% in small-cell lung cancer cells, and by 82%, 73%, 78% and 74% in human renal adenocarcinoma cells (119). *c-JUN* was reduced by vessel dilator, LANP, kaliuretic peptide and ANP by 43%, 31%, 61% and 35% in hepatocellular cancer cells, by 65%, 49%, 59% and 40% in small-cell lung cancer cells, and by 47%, 43%, 57% and 49% in renal cancer cells, respectively (119).

Thus, there appears to be a complex interaction of the four heart peptide hormones, *c-JUN, c-FOS* and MAPKs within cancer cells, as outlined in Figure 1, for in addition to the RAS–MEK-1/2–ERK-1/2 kinase cascade, another upstream regulator of *c-JUN* is JNK kinases, which phosphorylates c-JUN (115) and, in turn, JNK is inhibited (89%) by the four cardiac hormones (37). Both *c-FOS* and *c-JUN* are activated by STAT3 (114, 116) and the four heart peptides inhibit STAT3 (103). Thus, the cardiac hormones inhibit proliferative transcription factors (103) and by significantly inhibiting both c-FOS and *c-JUN*, and thus AP-1 protein, they most likely inhibit the transcription of various downstream genes and the transformation and progression of cancer regulated by AP-1 (105).

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