Heart Failure Stimulates Tumor Growth by Circulating Factors

BACKGROUND: Heart failure (HF) survival has improved, and nowadays, many patients with HF die of noncardiac causes, including cancer. Our aim was to investigate whether a causal relationship exists between HF and the development of cancer.

METHODS: HF was induced by inflicting large anterior myocardial infarction in APC\textsuperscript{min} mice, which are prone to developing precancerous intestinal tumors, and tumor growth was measured. In addition, to rule out hemodynamic impairment, a heterotopic heart transplantation model was used in which an infarcted or sham-operated heart was transplanted into a recipient mouse while the native heart was left in situ. After 6 weeks, tumor number, volume, and proliferation were quantified. Candidate secreted proteins were selected because they were previously associated both with (colon) tumor growth and with myocardial production in post–myocardial infarction proteomic studies. Myocardial gene expression levels of these selected candidates were analyzed, as well as their proliferative effects on HT-29 (colon cancer) cells. We validated these candidates by measuring them in plasma of healthy subjects and patients with HF. Finally, we associated the relation between cardiac specific and inflammatory biomarkers and new-onset cancer in a large, prospective general population cohort.

RESULTS: The presence of failing hearts, both native and heterotopically transplanted, resulted in significantly increased intestinal tumor load of 2.4-fold in APC\textsuperscript{min} mice (all $P<0.0001$). The severity of left ventricular dysfunction and fibrotic scar strongly correlated with tumor growth ($P=0.002$ and $P=0.016$, respectively). We identified several proteins (including serpinA3 and A1, fibronectin, ceruloplasmin, and paraoxonase 1) that were elevated in human patients with chronic HF (n=101) compared with healthy subjects (n=180; $P<0.001$). Functionally, serpinA3 resulted in marked proliferation effects in human colon cancer (HT-29) cells, associated with Akt-S6 phosphorylation. Finally, elevated cardiac and inflammation biomarkers in apparently healthy humans (n=8319) were predictive of new-onset cancer (n=1124) independently of risk factors for cancer (age, smoking status, and body mass index).

CONCLUSIONS: We demonstrate that the presence of HF is associated with enhanced tumor growth and that this is independent of hemodynamic impairment and could be caused by cardiac excreted factors. A diagnosis of HF may therefore be considered a risk factor for incident cancer.
Clinical Perspective

What Is New?

- New-onset cancer is prevalent in patients with heart failure, but it remains unclear whether the failing heart itself contributes to tumor growth.
- The current translational data provide for the first time direct proof that the presence of heart failure itself stimulates tumor formation, possibly via secreted factors.

What Are the Clinical Implications?

- Heart failure as a consequence of cancer treatment is an accepted phenomenon and has been widely studied.
- In current daily practice, there is nearly no awareness that new-onset cancer can develop in patients with heart failure, and the results of this study will spark interest in this hitherto unexplored disease combination.
- We speculate that it may be recommended to consider differential surveillance programs to screen patients with heart failure who are at risk for cancer development.
- Further studies of cardiac secreted factors and their potential utility as cancer biomarkers could help to risk-stratify patients with heart failure in terms of their cancer risk.

Heart failure (HF) is associated with substantial morbidity and high mortality. In the last decade, it has become increasingly apparent that mortality in HF is caused not only by cardiac complications and events. It is well known that HF is characterized by the presence of multimorbidity, and the extent of comorbid diseases strongly affects mortality. Indeed, recent registries and clinical trials observed that, compared with earlier trials, a larger percentage of patients with HF currently die of noncardiac causes. However, most attention has been on renal disease, diabetes mellitus, and atrial fibrillation; little attention has been paid to cancer development. In this study, we used 2 different mouse models. The first model included mice in which a myocardial infarction (MI) was induced in the donor mice (n=22) and the corresponding control sham-operated mice (n=10). Cardiac magnetic resonance imaging was performed as reported 1 week after operation to assess cardiac function. The second model was mice receiving either an MI or sham donor heart implanted into the cervical (neck) area. Donor mice (providing the donor heart) were subjected to an MI (n=17) or sham (n=7) operation 1 week before transplantation. Just before transplantation, cardiac function of the to-be-transplanted heart was assessed (in the donor mice) with echocardiography. In line with this hypothesis, recent epidemiological and case-control studies showed that patients with prevalent HF were more prone to developing incident cancer. In another community-based cohort, subjects with HF had an increased risk of developing cancer that was independent of age and sex. However, these studies are associative and cannot prove causality of the observed relation between prevalent HF and new-onset cancer. We therefore aimed to explore this possible causal relationship between HF and cancer development.

Methods

The data, analytical methods, and study materials will be made available to other researchers for purposes of reproducing the results or replicating the procedure for the in vitro and in vivo study. A request for the PREVEND data can be made at www.prevend.org.

Mouse Model

All animal experiments have been conducted with the C57BL/6J-ApcMin/J (APC<sup>min</sup>) mouse strain purchased from The Jackson Laboratory (Bar Harbor, ME). This strain is highly susceptible to spontaneous intestinal adenoma formation. Further details on the strain can be found in the online-only Data Supplement. All experimental procedures were performed in accordance with the European Union guidelines (DEC 6844, the Netherlands) and with the protocol established by the Austrian Federal Ministry of Science, Research and Economy (Austria) for the care and use of animals. Experiments were approved by the Austrian Ministry of Education, Science, and Culture (BMWF-66011_0063-II_10b_2010).

In Vivo Study Design

In this study, we used 2 different mouse models. The first model included mice in which a myocardial infarction (MI) was induced (n=22) and the corresponding control sham-operated mice (n=10). Cardiac magnetic resonance imaging was performed as reported 1 week after operation to assess cardiac function. The second model was mice receiving either an MI or sham donor heart implanted into the cervical (neck) area. Donor mice (providing the donor heart) were subjected to an MI (n=17) or sham (n=7) operation 1 week before transplantation. Just before transplantation, cardiac function of the to-be-transplanted heart was assessed (in the donor mice) with echocardiography. During a follow-up period of 6 weeks after heart transplantation (HTx) surgery, no mice were euthanized, excluding bias. Detailed methods are given in the online-only Data Supplement.

In Vitro Study Design

The human colorectal carcinoma cell line HT29 was grown and tested under various conditions. Detailed methods are given in the online-only Data Supplement.
General Population (PREVEND Study)

The PREVEND study (Prevention of Renal and Vascular End-Stage Disease) is a prospective, observational cohort study derived from the general population that comprises 8592 participants. This study was designed to monitor long-term development of cardiac, renal, and peripheral vascular end-stage disease. More details have been described previously. The study protocol conforms to the ethics guidelines of the 1975 Declaration of Helsinki and was approved by the institutional review board of the University of Groningen. Informed consent was obtained from all PREVEND participants. In this study, we measured the 5 candidate genes in 180 subjects (serving as controls for HF patients); furthermore in 8319 PREVEND subjects, we measured cardiac markers NT-proBNP (N-terminal pro-B-type natriuretic peptide; an established biomarker of HF), high-sensitivity troponin T, and MR-proANP (MR-pro-atrial natriuretic peptide); inflammation markers C-terminal proendothelin-1, MR-pro-adrenomedullin, high-sensitivity C-reactive protein, and procalcitonin; and neuroendocrine markers aldosterone, renin, and galectin-3.

Chronic Heart Failure (VitD-CHF Trial)

We used banked plasma from 101 patients with chronic HF who were enrolled in a previously published study (VitD-CHF [Study to Investigate the Effects of Vitamin D Administration on Plasma Renin Activity in Patients With Stable Chronic Heart Failure]; ClinicalTrials.gov identifier, NCT01092130). Patients ≥18 years of age, had a left ventricular ejection fraction (LVEF) <45%, and were treated with optimal HF medication. The study was approved by the institutional review board, and all patients provided written informed consent. Further details are provided in the online-only Data Supplement.

Statistical Analyses

Mouse Studies

Normally distributed variables are presented as mean±SD. Nonnormally distributed variables are expressed as medians (interquartile ranges). Experimental, clinical, and biochemical characteristics were compared across 2 groups with the 2-sample t test for continuous, normally distributed variables and the Wilcoxon rank-sum test for continuous, nonnormally distributed variables. We performed linear regression analyses to demonstrate the association between tumor load and either the amount of fibrosis or left ventricular function.

Analyses in PREVEND

The PREVEND study was set up to overselect participants with increased urinary albumin excretion (>10 mg/L). A designed-based statistical weighting was used to adjust for this over-selection, allowing conclusions to be made for the general population. As a first assessment, we performed cumulative hazard plots with the log-rank test after stratifying the population in tertiles based on NT-proBNP level. To study the association more deeply and including all the markers measured in the PREVEND study related to cardiac tissue, neuroendocrine markers, and inflammation, we used Cox proportional hazards regression models, first unadjusted and then adjusted for age, smoking, and body mass index. These covariates were selected because shared risk factors might bias our findings.

RESULTS

Assessment of Cardiac Function and Remodeling in Mice With HF After MI

To study the interaction between HF and tumor growth, we induced a large anterior MI to provoke HF. We studied APC(mn) mice, which are prone to developing intestinal polyps and tumors. One week after surgery, we performed cardiac magnetic resonance imaging (Figure 1A) to assess cardiac function. LVEF was markedly decreased after MI compared with sham-operated animals: 61% versus 32% (P<0.001; Figure 1B). Functional cardiac parameters in both groups are presented in Tables I and II in the online-only Data Supplement. MI-induced HF was associated with lower systolic blood pressure and elevated left ventricular end-diastolic pressure accompanied by increases in atrial and liver weights. Cardiac fibrosis was determined by Masson trichrome staining on cardiac tissue slides and quantified (Figure 1A) and was increased 3.2-fold in mice with HF (P<0.0001; Figure 1C). Related to the latter, genes associated with inflammation and fibrosis were significantly upregulated in HF on the mRNA, protein, and plasma levels (Figure 1E through 1H and Figure Ia through IJ in the online-only Data Supplement), with the most prominent increase for interleukin-6 (on both the mRNA and plasma levels) NPPA, the gene encoding ANP, an established surrogate HF marker, was >20-fold increased in HF compared with sham mice (Figure 1D; P<0.0001).

Assessment of Tumor Growth in MI-Induced HF

After 6 weeks, mice were euthanized, and intestinal tissue were harvested. The intestines were pinned down directly after euthanasia, and polyps were counted and measured. Significantly more (n=57 versus n=34; P<0.001; Figure 2A) and larger (1.69 mm versus 1.44 mm; P<0.001; Figure 2B) polyps were observed in APC(mn) mice with HF compared with sham-operated animals. We calculated overall tumor load, assuming a spherical morphology of the polyps, and demonstrated a 2.4-fold increase in HF mice (P<0.0001; Figure 2C). To further study the increased growth, we performed Ki-67 staining and found increased numbers of Ki-67–positive cells (54% in HF versus 44% in sham; P<0.05; Figure 2E). A well-described feature of this model is intestinal blood loss caused by polyp bleeding, result-
ing in anemia and splenomegaly and in a significantly higher spleen weight in HF compared with sham mice (P = 0.038; Figure 2D).

Association Between Cardiac Remodeling and Tumor Growth

To relate the changes in tumor growth to parameters of severity of HF, we performed linear regression analyses between tumor load and indexes of cardiac remodeling: fibrosis and LVEF. Both indexes were associated with tumor load (fibrosis: β = 0.51, P = 0.016; Figure 2G; and LVEF: β = −0.63, P = 0.002; Figure 2H).

Heterotopic HTx to Rule Out Hemodynamic Causes of HF-Induced Tumor Growth

To rule out whether hemodynamic impairment such as hypoperfusion (resulting from decreased systolic blood pressure and forward failure) or congestion (backward failure) in response to elevated filling pressures (with liver and/or gut congestion) would explain our initial observations, we conducted a second experiment. Here, we again inflicted MI in APCmin mice (donors), but this time, after 1 week, we performed heterotopic HTx, transplanting the (extra) infarcted heart (or sham-operated heart) into the cervical region of the recipient APCmin mice. This resulted in APCmin mice (receivers) with a normal native heart in situ, ensuring normal circulation, but with an additional heart, either with or without HF (according to whether the donor was subjected to MI or to sham operation). To assess the severity of MI, before transplantation, cardiac function was measured with echocardiography (Figure IIA in the online-only Data Supplement); cardiac parameters are presented in Table III in the online-only Data Supplement; cardiac parameters are presented in Table III in the online-only Data Supplement. Donor mice with HF exhibited severely impaired cardiac function before transplantation (LVEF, 61% versus 27%; P < 0.0001; Figure IIB in the online-only Data Supplement), which was comparable to our first experiments.
Six weeks after transplantation, we performed cardiac phenotyping of the transplanted (failing) heart, as described in the initial experiment. We again observed significant differences in fibrosis and inflammation (Figure IIC in the online-only Data Supplement and Figure 2E through 2H). In addition, an increased expression of NPPA (2.4-fold increase; \( P < 0.05 \), sham-operated transplanted hearts versus HF transplanted hearts). Although the changes between the initial and HTx experiments were both directionally comparable and significant for all tested markers, the overall changes were clearly less strong in the HTx model compared with the initial model. For instance, the changes in ANP were \( \approx 10 \)-fold less. This led us to assume that the transplanted hearts must be considered unloaded hearts and as a result produce and secrete fewer remodeling factors. The endogenous hearts of the recipient mice demonstrated no loss of function, as shown in Table III in the online-only Data Supplement, proving that our experimental design ensured normal systemic circulation.

**Assessment of Tumor Growth in Mice With Transplanted Hearts With HF**

A sacrifice protocol similar to that described before was performed, and intestinal polyps were counted and measured. Significantly more (55 versus 39; \( P < 0.01 \); Figure 3A) and larger (1.97 mm versus 1.51 mm; \( P < 0.01 \); Figure 3B) polyps were observed in mice receiving an HF heart transplant compared with mice receiving a sham-operated heart transplant. Again, the tumor load was calculated, and in this model, we also observed a significant 2.4-fold increase (\( P < 0.0001 \); Figure 3C), comparable to the initial experiment. To further validate our...
findings, we again showed increased numbers of KI-67–positive cells (54% versus 44%; \( P < 0.05 \); Figure 3E).

Finally, we also observed a significantly larger spleen (1.4-fold increase; \( P < 0.05 \); Figure 3D) in mice that received an HF heart transplant compared with sham. The other organs did not differ in weight between groups (Table IV in the online-only Data Supplement).

**Association Between Cardiac Remodeling and Tumor Growth**

Also in this experimental model, we performed linear regression analyses to determine the association between tumor load and indexes of cardiac remodeling. Here, we found comparable associations between tumor load and fibrosis (\( \beta = 0.89, P < 0.01 \); Figure 3G) and between tumor load and LVEF (\( \beta = -0.60, P = 0.002 \); Figure 3H).

**Exploration Strategy to Identify HF-Specific Secreted Proteins Capable of Enhancing Tumor Growth**

Our findings from the initial and HTx experiments suggest that the enhanced tumor formation in mice with HF is independent of hemodynamic factors and may be explained by secreted factors from the failing hearts. We present our hypothesis based on the in vivo findings in Figure 4, proposing that proteins may be excreted from failing hearts into the bloodstream and affect peripheral organs, here in particular the intestines, resulting in enhanced tumor development and growth.

We made use of the abundant biomedical literature that is available and set out to identify proteins secreted into the bloodstream in response to MI, focusing on articles reporting “shotgun proteomic” approaches of plasma samples after MI. Second, we ascertained which...
epitopes exist on intestinal tissue that theoretically can be bound or activated by these “cardiac” proteins. A detailed description of this methodology is provided in the online-only Data Supplement. Figure III in the online-only Data Supplement summarizes our literature findings. We identified 5 proteins with potential importance: α-1-antitrypsin (SerpinA1), α-1-antichymotrypsin (SerpinA3), fibronectin, ceruloplasmin, and paraoxonase 1.

Validation of Myocardial Gene Expression of Identified Secreted Factors

First, to validate whether the identified candidates are upregulated in the left ventricular tissue of mice with HF (compared with sham), we performed quantitative polymerase chain reaction for both our initial and the HTx studies. In our discovery study, we demonstrated a significantly increased left ventricular expression for all 5 genes (sham versus HF; all \( P < 0.05 \)). Then, we assayed the same genes in the HTx study, validating 3 of the 5 genes: SerpinA3, fibronectin, and paraoxonase 1 (Figure IV in the online-only Data Supplement).

Proliferation of HT-29 Cells Resulting From the Addition of the Secreted Proteins

To investigate whether the identified proteins actually exert proliferative effects on colorectal cells, we performed in vitro experiments with HT-29 cells, which are a commonly used cell type of colorectal cancer. HT29 cells were grown and seeded in DMEM and, before the experiment, starved and cotreated with Suramin to halt cell proliferation. After 48 hours, cells were treated with 0.1% FCS (negative control), 10% FCS (positive control), or the identified candidate proteins. To assess proliferation, we used 3 assays: We measured PNCA, a gene indicative of cell proliferation, and expressed the results as a ratio to the positive and negative controls, and we performed staining with 5-ethynyl-2′-deoxyuridine EdU+ and KI-67+. SerpinA3 and SerpinA1 resulted in increased proliferation (33% and 21%, respectively). The other proteins provoked no significant differences in proliferation rate compared with low FCS concentrations (Figure VA in the online-only Data Supplement). We confirmed the proliferative effects of SerpinA3 by showing increased PCNA expression and EdU+ and KI67 staining after the addition of a different SerpinA3 concentration to HT-29 cells. SerpinA3 consistently demonstrated proliferation evidenced by these different assays (Figure VB through VD in the online-only Data Supplement).

Activation of Growth Pathways in Colon Cells in Response to SerpinA3

To further elucidate potential cellular mechanisms by which SerpinA3 results in proliferation, we stimulated HT-29 cells with SerpinA3 as described above and performed Western blot analyses for the Erk1/2 and Akt signal transduction pathways that have been linked to SerpinA3 stimulation. Akt phosphorylation and a downstream target, ribosomal protein S6 (rpS6), which marks cell growth, were both significantly phosphorylated on SerpinA3 stimulation, whereas Erk1/2 was not targeted (Western blots and a simplified graphical scheme are displayed in Figure 5), suggesting that SerpinA3 provokes tumor growth via the Akt pathway.

Cardiac Secreted Proteins Are Elevated in Plasma of Human Patients With HF

To explore whether we could translate our in vivo and in vitro findings to the human situation, we measured all 5 candidate proteins in 180 healthy subjects enrolled in the PREVEND study and in 101 patients with chronic HF. We used human ELISAs that have been validated (online-only Data Supplement). Baseline characteristics are presented in Table 1. Indeed, we demonstrated that plasma levels of all 5 proteins were 30% to 100% upregulated (all \( P < 0.001 \)) in patients with HF, which underscores that these proteins indeed are related to the presence of HF (Figure 6).

Cardiac and Inflammatory Markers Are Associated With New-Onset Cancer

In 8319 subjects of the PREVEND, a community-based cohort study with middle-aged participants, we evalu-
ated the predictive value of cardiac markers, including NT-proBNP, an established biomarker of HF, high-sensitivity troponin T, and MR-proANP. We previously have shown that NT-proBNP in this cohort strongly predicts new-onset HF. In addition to these markers, we investigated inflammation-related proteins, including C-terminal proendothelin-1, MR-pro-adrenomedullin, high-sensitivity C-reactive protein, and proccalcitonin. Further neuroendocrine markers such as aldosterone, renin, and galectin-3 were analyzed. New-onset cancer cases were provided through the nationwide network and registry of histopathology and cytopathology in the Netherlands (PALGA) and were reviewed and adjudicated by an independent committee. During a median follow-up of 11.5 years, 1132 subjects (13.1%) were diagnosed with cancer, 132 (11.7%) with colorectal cancer. We performed Kaplan-Meier analysis for both all-cause cancer and colorectal cancer according to tertiles of NT-proBNP levels. Compared with subjects in tertile 1 (low NT-proBNP), subjects in tertiles 2 and 3 (higher and highest NT-proBNP) had an increased risk of developing all-cause cancer and colorectal cancer (both P<0.0001; Figure 7). Next, we performed Cox proportional hazard regression analyses to adjust for common risk factors of new-onset cancer. These data show that the association of cardiac and inflammatory biomarkers with all-cause cancer remains, also after adjustment for age, sex, smoking status, and body mass index (Table 2), whereas the significant association for colorectal cancer was lost after correction, possibly because of limited power (Table V in the online-only Data Supplement). The neuroendocrine biomarkers were not associated with all-cause cancer or colon cancer, except for galectin-3 (only unadjusted). To extrapolate these findings to other types of cancer, we repeated these analyses for breast, lung, skin, urologic, hematologic, and male and female reproductive system cancers (Tables XI through XII in the online-only Data Supplement). Both cardiac and inflammation biomarkers were (unadjusted and adjusted) associated with new-onset cancer.

Figure 5. Growth pathways in colon cells in response to SerpinA3.
A, Western blot analysis of total (T) and phosphorylated (P) Akt, rpS6, and Erk1/2. B, Quantification of Akt, rpS6, and ERK 1/2 phosphorylation. Data are presented as mean±SEM. *P<0.05. C, Scheme of Akt and rpS6 phosphorylation that marks cell growth resulting from SerpinA3 stimulation in HT-29 cells, whereas Erk1/2 was not targeted.
lung and male reproductive system cancer, and cardiac biomarkers were associated only with female reproductive system cancers. It is clear that these associations are exploratory, but they provide additional suggestions for the relation between HF and incident cancer.

**DISCUSSION**

We demonstrate for the first time a causal relationship between HF and tumor growth. First, we explored this novel paradigm by creating MI-induced HF in a murine model of precancerous polyps, and experimental HF resulted in increased tumor formation and accelerated tumor growth. Second, we corroborated our results in an independent model in an independent laboratory using a heterotopic murine HTx model, and we validated our initial results while ruling out hemodynamic impairment as a cause. To probe our hypothesis that cardiac secreted factors of the failing heart could be responsible, we conducted a literature search from databases of myocardial secreted proteins and connected the candidates to databases of proteins previously associated with new-onset colorectal cancer. We then validated the candidate secreted proteins in vitro, in vivo, and in human studies, and we identified SerpinA3 as the most robust and promising culprit. We explored the potentially contributory involvement of inflammatory factors. Finally, we provided human validation by showing that cardiac markers, including NT-proBNP, and inflammatory markers, including C-reactive protein, were associated with the prediction of new-onset cancer.

In the past decade, significant progress has been made in the understanding of HF development in cancer patients. Recent position statements of the American Heart Association\(^{20}\) and the European Society of Cardiology\(^{21}\) have described this field in great detail. However, the reverse, that is, cancer in the setting of HF, has received far less attention. Lately, epidemiological data have emerged that patients with HF are at higher risk to be diagnosed with and/or to die of cancer compared with age-matched subjects without HF.\(^{8}\) This fits the modern face of HF in that mortality is no longer caused solely by cardiovascular death\(^{3}\) but is largely the result of other causes. In a study with nearly 800 patients with HF who were prospectively followed up for 5 years, cancer (next to stroke) was the second most important predictor of mortality, with a 2.5-fold increased risk in patients with HF with preserved ejection fraction.\(^{4}\) Comparable results were observed in another study comprising 2843 patients with HF with preserved ejection fraction and 6599 with HF with reduced ejection fraction with a 2-year follow-up.\(^{7}\) In a case-control study pairing 961 patients with incident HF and subjects without HF, patients with HF had a 68% higher risk for incident cancer, which appeared to progressively increase over time.\(^{8}\) Supporting evidence comes from another study that described that circulating levels of the NT-proBNP and high-sensitivity troponin T, markers of cardiac stretch and injury, were elevated in patients with cancer, already before the induction of any cardiotoxic anticancer therapy. These markers were related to all-cause mortality, suggesting that subclinical myocardial damage may be present in patients with cancer.\(^{22}\) Before this report, 1 small prospective study reported that NT-proBNP predicts future cancer develop-

| Table 1. Baseline Characteristics of the Patients With Heart Failure and Healthy Subjects (PREVEND) |
|-----------------|-----------------|-----------------|
| Variable        | Patients With Heart Failure (n=101) | PREVEND (n=8319) |
| Age, mean±SD, y | 64±10           | 49±13           |
| Male, n (%)     | 93 (93)         | 4127 (50)       |
| New York Heart Association class II/III, n | 89/11 | NA |
| Diabetes mellitus, n (%) | 14 (14) | 131 (2) |
| Current smoking, n (%) | 22 (22) | 3686 (45) |
| Left ventricular ejection fraction, mean±SD, % | 35±8 | ND |
| Systolic blood pressure, mean±SD, mmHg | 118±18 | 129±20 |
| Diastolic blood pressure, mean±SD, mmHg | 72±12 | 74±10 |
| Serum creatinine, mean±SD, μmol/L | 90±18 | 84±20 |

ND indicates not determined; and PREVEND, Prevention of Renal and Vascular End-Stage Disease.

**Figure 6. Human plasma levels of the identified candidate proteins.**

Plasma concentration of candidate factors in plasma from healthy subjects and patients with heart failure (HF). Data are presented as mean±SEM. PON1 indicates paraoxonase 1. ****P<0.0001.
opment in patients with coronary artery disease. Independent, circumstantial evidence that strengthens our hypothesis was provided by large randomized clinical trials with HF medication, the SOLVD trial (Studies of Left Ventricular Dysfunction) of enalapril versus placebo and the CHARM trial (Candesartan in Heart Failure: Assessment of Mortality and Morbidity) of candesartan versus placebo, in which a signal toward more cancer was observed in patients on active treatment. However, the excess incidence of cancer in this setting was suggested to be the potential consequence of competing risk; that is, if one reduces HF-related mortality, it has been assumed that cancer will have risen and that cancer-related mortality “takes over” from HF.

Our data suggest something different: The presence of a failing heart per se might contribute to tumor progression and formation. It is clear that tumorigenesis is a complex, multistage process characterized by several features, including resistance to growth inhibitors, autonomous proliferation independent of normal growth factor control, replication without limit, evasion of apoptosis, tissue invasion, and formation of metastases, with supportive growth of matrix and enhanced angiogenesis. These different biological processes are influenced by numerous different signal transduction pathways. Our identified proteins are involved in many of these processes, as discussed later, but given the complexity of this process, additional factors associated with HF also likely play a role.

Of our panel of candidate proteins, SerpinA3 emerged consistently as a factor that is increased in HF, with proliferative effects in vitro. SerpinA3, known to be associated with cardiac remodeling and matrix turnover, has also been investigated in relation to cardiac

### Table 2. Hazard Ratio for New-Onset Cancer per Biomarker Doubling

<table>
<thead>
<tr>
<th>Biomarker</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P Value</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>P Value</th>
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<td>N-terminal pro-B-type natriuretic peptide</td>
<td>1.39</td>
<td>1.32–1.46</td>
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<td>High-sensitivity troponin T</td>
<td>1.61</td>
<td>1.52–1.69</td>
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<td>1.02–1.19</td>
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<td>MR-pro-atrial natriuretic peptide</td>
<td>1.80</td>
<td>1.66–1.95</td>
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<td>Procalcitonin</td>
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CI indicates confidence interval.  
*Model 1 was adjusted for age, smoking, and body mass index.

COX proportional hazard analyses of doubling per biomarker level and the risk for new-onset cancer.
HF and donor humans, SerpinA3 was also identified as one of strongest regulated genes. Nevertheless, elevated SerpinA3 is associated with a worse prognosis and increased migration and invasion in melanoma. Finally, both SerpinA3 and SerpinA1 have been proposed as markers of tumor progression of adenoma into carcinoma and are linked to breast, prostate, and liver cancer. Collectively, SerpinA3 could directly link HF and cancer via its pleiotropic effects because it acts as an acute-phase protein and is related to systemic inflammation. We now extend these previous findings by showing that SerpinA3 is produced in murine HF, is elevated in the plasma of human patients with HF, and stimulates proliferation of colon tumor cells via an Akt-dependent pathway.

Fibronectin is an established central player in the cardiac extracellular matrix during cardiac remodeling and other physiological circumstances and is strictly regulated, for example, by the renin-angiotensin-aldosterone system. As in HF, extracellular matrix is important for tumor integrity and growth. Fibronectin has been implicated as a proangiogenic factor. Fibronectin acts on tumor-associated fibroblasts and enhances tumor growth and vasculature, stimulating expression of proangiogenic factors. Indeed, fibronectin has been proposed as a possible target to prevent angiogenesis because of its interaction with integrin receptors. Circulating fibronectin levels have been significantly elevated in patients with colorectal cancer compared with controls, and serum fibronectin levels rose further with cancer progression. Paraoxonase 1 expression and secretion in lung cancer have been shown to be pro-oncogenic and supported metastatic progression by decreasing the G1/S ratio and cell senescence. Serum paraoxonase 1 activity is increased in patients with colon cancer compared with control subjects. In a large Finnish registry of nearly 40,000 subjects, the overall incidence of cancer was positively associated with serum ceruloplasmin levels. The strongest association was observed in patients with lung cancer and in male patients. In breast cancer studies, it was hypothesized that it can be used as a biomarker of disease progression or as a marker for response to therapy. Recently, SARI, a direct ceruloplasmin target, has been studied in colon cancer and inhibits angiogenesis and tumor growth.

In addition to the 5 proteins that were discovered with our bio-informatic approach, the importance of inflammation as a shared pathway in HF and cancer must be considered, especially because the interleukin-1β antibody canakinumab was recently shown to decrease new-onset cancer in CANTOS (Canakinumab Antiinflammatory Thrombosis Outcome Study). In our mouse studies, we also observed clear increases in interleukin-6 levels and strong associations between the inflammatory markers C-reactive protein and MR-pro-adrenomedullin and incident cancer in the human (PREVEND) study.

The relation between cardiac secreted markers and the levels that can be measured systemically is complex. We show that for cardio-specific proteins such as ANP, this is straightforward: Increased cardiac production is reflected by increases in plasma levels (Figure IF through IH in the online-only Data Supplement). There are no well-validated ELISAs for mouse plasma for our candidate proteins. We have measured the HF marker NT-proANP, the fibrosis marker tissue inhibitor of metalloproteinases-1, and the inflammatory marker interleukin-6 for which validated mouse ELISA assays are available. The relations appear straightforward, although we cannot claim that this will also be true for the other proteins.

We acknowledge this as a limitation of our study, and although these results support the idea that elevated cardiac expression can confer effects on distant organs and tumors via plasma proteins, further research is required to solve this complex intertissue interaction.

Furthermore, in this study, we studied a post-MI model of HF, which is characterized by ischemia, cell death, and a large fibrotic scar, which likely are associated with a distinct proteomic signature. HF caused by other (nonischemic) pathogeneses will likely be characterized by another set of secreted proteins, which may cause differential effects on tumor growth. Future studies should address whether the pathogenesis of HF is important in this regard.

Another form of (acute) HF is stress cardiomyopathy (also called Takotsubo). An increased prevalence of malignancies has been observed by Sattler et al in patients with Takotsubo cardiomyopathy, both at the initial diagnosis and during follow-up. It has been hypothesized that this may originate from a common pathway of the 2 conditions, especially the catecholamine excess in cardiovascular disease and cancer. Yalta and Yalta
proposed that malignant diseases (or as-yet undiagnosed malignancy) might be the trigger for Tako-Tsubo. However, no molecular pathways have yet been discovered to definitely link these 2 disease modalities to each other.

Clearly, cancer as a comorbid condition in HF is very serious, and not surprisingly, cancer increases the mortality in HF. Do our data imply that cancer surveillance might be incorporated into the management of patients with HF? In an additional exploratory analysis of the screening for new-onset cancer, we investigated whether subjects enrolled in the PREVEND study who developed HF before 55 years of age (which in the Netherlands is the age when surveillance for colon cancer starts) were in fact at higher risk for developing cancer compared with patients who develop HF after 55 years of age. We observed that patients who developed HF before the age of 55 have a significantly higher risk of developing cancer compared with those without HF (hazard ratio, 2.43; 95% confidence interval, 1.33–4.43; P=0.004), whereas there was no difference between patients with or without HF after 55 years of age (hazard ratio, 1.05; 95% confidence interval, 0.84–1.33; P=0.636). This observation suggests (but does by no means prove) that people who are not yet eligible for screening because of “young age” might benefit from early screening for colorectal cancer once they develop HF.

In summary, our data show that the presence of HF is associated with increased formation and accelerated tumor growth in a mouse model of colon polyps. We identify several myocardial markers with established effects on tumor growth that we demonstrate are chronically elevated in human HF. One of our most promising candidate proteins might also be targeted for therapy, for example, with MRAs. Our preclinical and clinical data strengthen the link between HF and incident cancer. We propose that this might have implications for screening programs for new-onset cancer.

**Strengths and Limitations**

We conducted 2 independent laboratory studies with mice using different operating techniques and in different laboratories. All analyses were done blinded, and we did not exclude animals. Thus, this study conforms to the Animal Research: Reporting of In Vivo Experiments criteria for stringent methodology in animal studies. Clearly, the mouse APC \(^{min}\) model is a model of colon cancer formation, with all limitations, and does not allow extrapolation to other tumor types. Our current proteomic approach was not based on the in vivo models used in this study but rather on post-MI proteomic studies published by others. Because the effects of the initial study and the HTx study were comparable with respect to tumor growth, additional proteomic analyses might be instrumental in identifying which proteins exert the strongest effects on tumor growth.

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