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Serum Concentrations of Fibroblast Growth Factor 2 Are Increased in HIV Type 1-Infected Patients and Inversely Related to Survival Probability

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ABSTRACT

HIV-1-infected patients develop a generalized vasculopathy that is clinically most evident as Kaposi's sarcoma (KS), a multifocally appearing endothelial cell-derived tumor. Fibroblast growth factor 2 (FGF-2) is a potent autocrine and paracrine mitogen of endothelial cells and has been implicated in the cell proliferation and angiogenesis observed in KS. Here we determined by ELISA the FGF-2 serum concentrations in different clinical groups of HIV-1-infected patients. AIDS-KS patients (n = 53) and HIV-1-infected patients without KS (n = 39) revealed significantly increased FGF-2 serum concentrations (median, 4.5 and 4.6 pg/ml, respectively), as compared with the healthy control group (n = 22; median, 2.2 pg/ml; $p < 0.01$). FGF-2 concentrations were highest in untreated HIV-1-infected patients (median, 8.6 pg/ml) and were significantly decreased in patients undergoing antiretroviral therapy (AZT-median, 4.5 pg/ml; HAART-median, 2.5 pg/ml; $p < 0.01$). In addition, FGF-2 serum concentrations above 5.2 pg/ml were associated with a statistically significant higher risk of death in HIV-1-infected patients. Multivariate analysis showed that this effect is independent of CD4 levels, localization of KS (cutaneous or visceral), AIDS-defining opportunistic diseases, and therapy. Circulating FGF-2 may contribute to AIDS-associated vasculopathy and may be a sensitive and easily accessible surrogate marker to determine the survival time of HIV-1-infected patients and the efficacy of antiretroviral therapy.

INTRODUCTION

ACTIVATION AND DYSFUNCTION of endothelial cells are important disease parameters in human immunodeficiency virus type 1 (HIV-1)-infected patients, as indicated by the microangiopathy syndrome,[1,2] blood–brain barrier defects,[3] and Kaposi's sarcoma (KS), which affects almost 20% of homosexual men infected with HIV-1.[4] In addition, structural disruptions of the aortic endothelium, concomitant with endothelial cell activation and increased adhesion of mononuclear cells, have been detected in almost 80% of HIV-1-infected patients.[5] HIV-1 does not commonly infect endothelial cells. Therefore, circulating systemically active HIV-1 proteins or cell-encoded factors with endothelial cell tropism may trigger AIDS-associated vasculopathy.

Fibroblast growth factor 2 (FGF-2) is a potent activator of endothelial cells in tumor angiogenesis and inflammation.[6] In addition, experimental evidence has been provided that FGF-2 is implicated in the development of KS: FGF-2 is highly expressed in KS spindle cells *in vitro* and *in vivo*,[7–9] induces the formation of KS-like lesions in mice, and acts with synergistic activity when combined with vascular endothelial cell growth factor (VEGF) or with HIV-1 Tat protein.[8,10,11] Here we show that the serum concentration of FGF-2 is (1) significantly increased in HIV-1-infected patients irrespective of KS, (2) reduced to normal levels by antiretroviral therapy, and most importantly (3) correlates with an increased risk of death. These results indicate that circulating FGF-2 may be a useful surrogate marker to determine the efficacy of antiretroviral therapy and to predict the survival time of HIV-1-infected patients.

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MATERIALS AND METHODS

Patients

Serum samples were obtained from 39 HIV-1-infected male patients without KS (HIV-1 group) ranging in age from 25 to 61 years with a median of 37 years, and from 53 HIV-1-positive male patients with KS (aged from 22 to 61 years; median, 39 years) (AIDS–KS group). Thirty-six patients of the HIV-1 group and 42 patients of the AIDS–KS group were undergoing conventional antiretroviral therapy with one or two nucleoside reverse transcriptase inhibitors (NRTIs: azidothymidine [AZT], didanosine [ddI], zalcitabine [ddC], stavudine [d4T]), and 3 patients of the HIV-1 group and 3 patients of the AIDS–KS group were treated with highly active antiretroviral therapy (HAART) (two NRTIs and one protease inhibitor). Eight AIDS–KS patients were not enrolled in antiretroviral treatment. In addition, 11 of the AIDS–KS patients (8 treated with AZT and 3 treated with HAART) were treated with liposomal doxorubicin.[12] Control sera were collected from 22 healthy male individuals ranging in age from 24 to 74 years (median, 57 years) without known neoplasm, recent trauma, or surgery. All individuals enrolled in the study were white. Informed consent to take the blood samples was obtained from all of the individuals.

Enzyme-linked immunosorbent assay

Concentrations of FGF-2 in undiluted sera (stored at −70°C) were determined with a commercially available enzyme-linked immunosorbent assay (ELISA) kit (Quantikine high sensitivity; R&D Systems, Wiesbaden-Nordenstadt, Germany) according to the manufacturer instructions. All analyses and calibrations were carried out in duplicate and each plate included recombinant human FGF-2 standards. The intensity of the chromogenic reactions was evaluated spectrophotometrically at 490 nm, using an ELISA reader (Dynatech Laboratories, Chantilly, VA) with a correction filter at 630 nm.

Statistical analysis

Statistical analysis to evaluate levels of FGF-2 in different groups was performed by the Mann–Whitney U test. Factors associated with time to death were assessed by Cox univariate and multivariate regression proportional hazard models. Estimates of survival distribution were done by the Kaplan–Meier analysis and the log-rank test. A p value lower than 0.05 was considered to be significant. All analyses were performed with the statistical software STATA (Stata, College Station, TX).

RESULTS

FGF-2 concentrations were measured in the sera of 53 HIV-1-infected patients with KS (AIDS–KS group), 39 HIV-1-infected patients without KS (HIV-1 group), and 22 uninfected healthy control persons (control group), and were found to be significantly elevated both in the HIV-1 group (range from 0.4 to 22 pg/ml; mean, 6.3 ± 5.5 pg/ml; median, 4.6 pg/ml; $p < 0.01$ [Fig. 1, HIV-1]) and in the AIDS–KS group (0.66 to 34.6 pg/ml; mean, 7.8 ± 8.3 pg/ml; median, 4.5 pg/ml; $p < 0.01$ [Fig. 1, AIDS–KS]) as compared with the control group (0 to 5.2 pg/ml; mean, 2.6 ± 1.6 pg/ml; median, 2.2 pg/ml [Fig. 1, control]). The AIDS–KS and the HIV-1 groups did not reveal significantly different FGF-2 concentrations ($p = 0.78$). In the control group the highest FGF-2 concentration measured was 5.2 pg/ml. Twenty-four patients (45.3%) of the AIDS–KS group and 18 patients (46%) of the HIV-1 group had FGF-2 concentrations above this threshold value.

Analysis of the HIV-1-infected patients (AIDS–KS and HIV-1 group, $n = 92$) with respect to antiretroviral therapy (AZT,

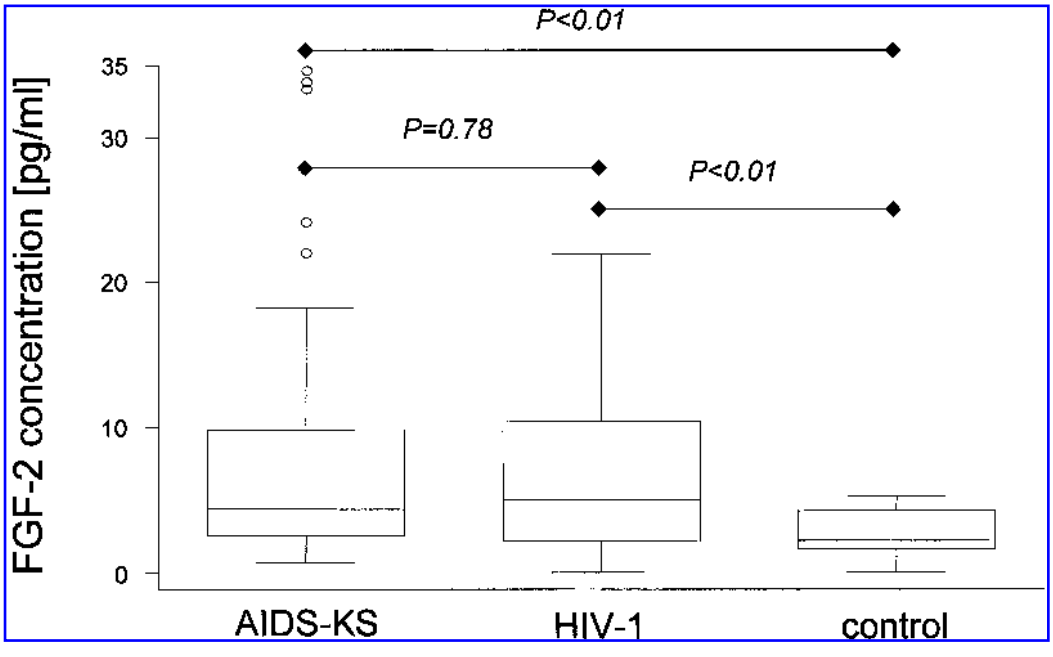

FIG. 1. Box-and-whisker plots of FGF-2 serum concentrations in AIDS–KS patients, HIV-1-infected patients without KS, and control subjects. Statistical significance of differences was analyzed by the Mann–Whitney U test.

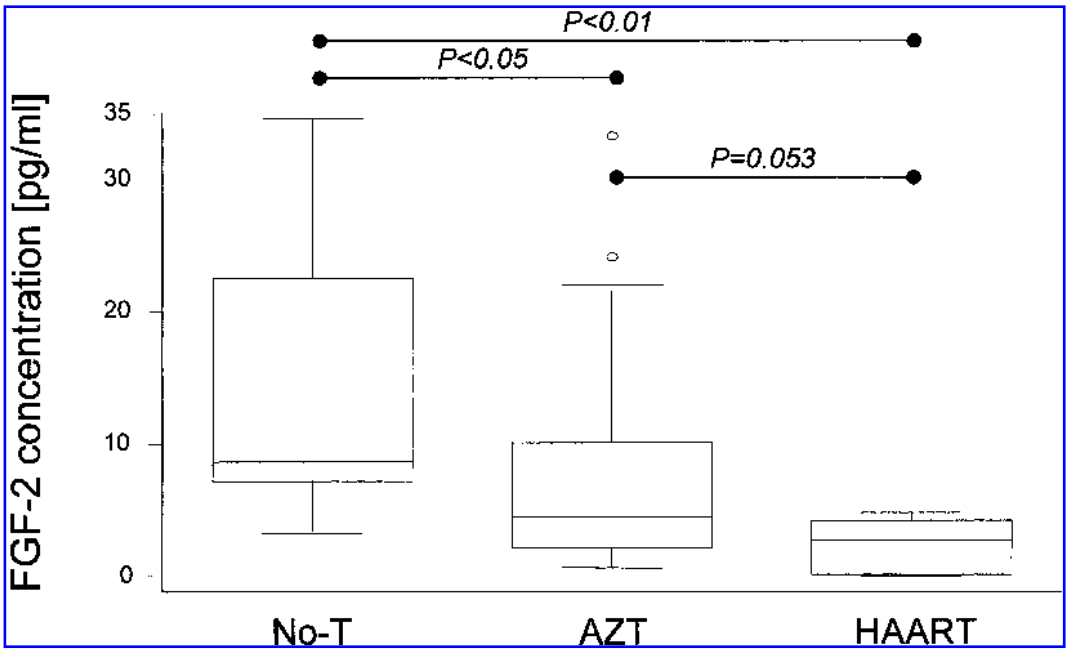

FIG. 2. Influence of antiretroviral therapy on FGF-2 serum concentrations in HIV-1-infected patients: HIV-1-infected patients without treatment (No-T), treated with one or two NRTIs (AZT), or with two NRTIs and one protease inhibitor (HAART). Statistical significance of differences was analyzed by the Mann–Whitney U Test.

HAART) revealed that patients treated with AZT (range, 0.66 to 33.3 pg/ml; mean, 6.8 ± 6.3 pg/ml; median, 4.5 pg/ml; $n = 78$) or with HAART (range 0.4 to 4.8 pg/ml; mean, 2.4 ± 2 pg/ml; median, 2.5 pg/ml; $n = 6$) had significantly lower FGF-2 serum concentrations as compared with untreated HIV-1-infected patients (No-T) (from 3.2 to 34.6 pg/ml; mean, 14.4 ± 12.5 pg/ml; median, 8.6 pg/ml; $n = 8$) (AZT, $p < 0.05$; HAART, $p < 0.01$) (Fig. 2). The difference in FGF-2 serum concentrations in HAART-treated and AZT-treated patients was at the borderline of statistical significance ($p = 0.053$).

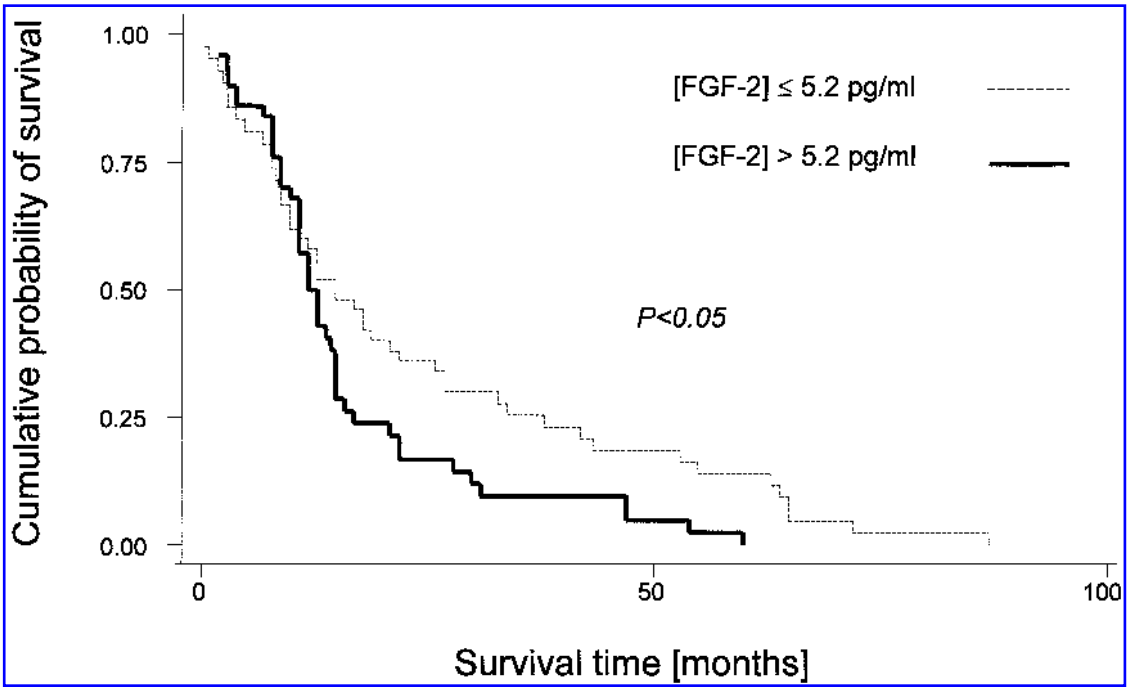

FIG. 3. Cumulative probability of survival of HIV-1-infected patients with FGF-2 serum concentrations above or below 5.2 pg/ml. The survival probability was assessed by Kaplan–Meier analysis. Statistical significance of difference was analyzed by the log-rank test.

Treatment with liposomal doxorubicin (11 patients of the AIDS–KS group) did not affect FGF-2 serum concentrations (data not shown).

In HIV-1-infected patients an increase in the FGF-2 concentration of 1 pg/ml correlated with a statistically significant increase in the crude relative hazard (RH) of death of 5.5% (95% confidence interval [CI], 2.1–8.9%; $p < 0.01$) in Cox univariate regression proportional hazard analysis. This finding remained statistically significant in Cox multivariate regression analysis adjusting simultaneously for $CD4^+$ lymphocyte numbers, site of KS lesions (cutaneous or visceral), AIDS-defining opportunistic diseases and therapy (RH of 3.3% for each increase of 1 pg/ml in FGF-2 serum levels; 95% CI, 0.2–7.0%; $p < 0.05$). Since all control individuals showed FGF-2 levels below 5.2 pg/ml, this value was chosen as the cutoff to determine whether increased concentrations of circulating FGF-2 may be associated with an increased risk of death. The Kaplan–Meier estimate of survival distribution for HIV-1-infected patients demonstrated that FGF-2 concentrations above 5.2 pg/ml were associated with a significantly reduced survival probability (log-rank test, $p < 0.05$) (Fig. 3).

DISCUSSION

Here we showed that serum concentrations of the angiogenic protein FGF-2 are increased in HIV-1-infected patients and that this correlates with a significantly reduced probability of survival of these patients. FGF-2 is produced by several different cell types, including T lymphocytes, macrophages, granulocytes, and activated endothelial cells,[13–18] and is secreted from the cells by a signal peptide-independent pathway, possibly mediated by an Na^+,K^+-ATPase.[19] Extracellularly FGF-2 is bound to heparan sulfate proteoglycans (HSPG) from which it is released by degradation of extracellular matrix (ECM) components.[20] Inflammatory cytokines such as interleukin 1 (IL-1), tumor necrosis factor (TNF), and interferon γ (IFN-γ), which are all present in increased concentrations in the serum of HIV-1-infected patients,[21–23] have been shown to induce FGF-2 expression in endothelial and KS cells. Moreover, the HIV-1 Tat protein by its basic domain is able to release FGF-2 from the ECM.[24,25] These mechanisms may explain the increased concentrations of FGF-2 in the serum of HIV-1-infected patients and, vice versa, suggest that the reduction of FGF-2 serum concentrations by antiretroviral therapy may reflect both the normalization of the inflammatory cytokine concentrations and the repression of circulating HIV-1 Tat protein in treated patients. In this framework, FGF-2 may be a sensitive and easily accessible surrogate marker indicating the therapeutic repression of AIDS pathogenic factors such as the HIV-1 Tat protein, for which at present no appropriate serological assay is available.

From our data it cannot be concluded whether the increase in FGF-2 serum concentration in HIV-1-infected patients is causative or a consequence of decreased probability of survival during AIDS progression. However, FGF-2 is also increased and correlates with risk of mortality in patients with pediatric high-grade glioma, various solid tumors, and non-Hodgkin's lymphoma.[26–28] In these diseases FGF-2 serum concentrations reflect angiogenic activity of the tumors that is key for tumor growth and metastatic spread and by this increases tumor malignancy and mortality of the patients. Furthermore, it has been shown that FGF-2 inhibits the adhesion of activated natural killer cells to tumor vessel endothelium, which may protect tumor cells from the immune response.[29] Therefore, FGF-2, through its vasculotropic activity, may have direct impact on the mortality of tumor patients.

In HIV-1-infected patients, in addition to FGF-2, VEGF and inflammatory cytokines are also present in increased serum concentrations.[21–23,30] In combination with the HIV-1 Tat protein all these factors, including FGF-2, reveal synergistic activity on endothelial cells, affecting proliferation, migration, adhesiveness of monocytes to these cells, angiogenesis, and vascular permeability.[4] Therefore, under the vasculoactive conditions of HIV-1 infection circulating FGF-2 may significantly contribute to edema, blood–brain barrier defects, and KS development, and possibly may also induce generalized vascular malfunctions that may contribute to reduced survival of HIV-1-infected patients and to the progression of AIDS.

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