Personalized Medicine and Imaging

Serum Interleukin-8 Reflects Tumor Burden and Treatment Response across Malignancies of Multiple Tissue Origins

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Abstract

Purpose: Interleukin-8 (IL8) is a chemokine produced by malignant cells of multiple cancer types. It exerts various functions in shaping protumoral vascularization and inflammation/immunity. We evaluated sequential levels of serum IL8 in preclinical tumor models and in patients to assess its ability to estimate tumor burden.

Experimental Design: IL8 levels were monitored by sandwich ELISAs in cultured tumor cells supernatants, tumor-xenografted mice serum, and in samples from 126 patients with cancer. We correlated IL8 serum levels with baseline tumor burden and with treatment-induced changes in tumor burden, as well as with prognosis.

Results: IL8 concentrations correlated with the number of IL8-producing tumor cells in culture. In xenografted neoplasms, IL8 serum levels rapidly dropped after surgical excision, indicating an accurate correlation with tumor burden. In patients with melanoma ($n = 16$), renal cell carcinoma (RCC; $n = 23$), non–small cell lung cancer (NSCLC; $n = 21$), or hepatocellular carcinoma (HCC; $n = 30$), serum IL8 concentrations correlated with tumor burden and stage, survival (melanoma, $n = 16$; RCC, $n = 23$; HCC, $n = 33$), and objective responses to therapy, including those to BRAF inhibitors (melanoma, $n = 16$) and immunomodulatory monoclonal antibodies (melanoma, $n = 8$). IL8 concentrations in urine ($n = 18$) were mainly elevated in tumors with direct contact with the urinary tract.

Conclusions: IL8 levels correlate with tumor burden in preclinical models and in patients with cancer. IL8 is a potentially useful biomarker to monitor changes in tumor burden following anticancer therapy, and has prognostic significance. *Clin Cancer Res; 20(22); 5697–707. ©2014 AACR.*

Introduction

Malignant cells and tumor stroma from diverse tissue origins biosynthesize and secrete the interleukin-8 (IL8) chemokine (1,2), rendering this polypeptide a potential biomarker in cancer.

IL8 (CXCL8) is a member of the CXC chemokine family with the ELR motif (3). It was originally identified as a proinflammatory chemotactic factor for neutrophils (4). Already known to enhance tumor cell growth and promote angiogenesis (5,6), more recently, IL8 has been proposed to be a potent protumoral factor. In breast cancer and in glioblastoma, it has recently been suggested that IL8 directly favors cancer stem cells (7,8).

Because of its potent proinflammatory properties, IL8 is tightly regulated, and its expression is low or undetectable in normal tissue. IL8 expression is primarily regulated by activator protein 1 (AP1) and nuclear factor-κB (NF-κB) as transcription factors. Different stimuli have been shown to upregulate IL8 expression including inflammatory signals (i.e., tumor necrosis factor-α and IL1β) or environmental stresses (i.e., hypoxia and chemotherapy agents). In contrast, steroid hormones (corticosteroids, androgens, and estrogens) have been described to downregulate IL8 production (9).

Multiple mechanisms are apparently involved in the protumoral actions of IL8. These include direct effects on

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Translational Relevance

Tumor burden monitoring is critical in the assessment of response to treatment and the management of patients with cancer. Radiologic imaging is the most widely used and reliable technology for this purpose, but has important limitations when evaluating tumor response to antiangiogenic drugs and immunotherapy. Serum tumor markers are also useful in carcinomas of certain tissue origins. We found that interleukin-8 (IL8) serum concentrations accurately reflect viability of tumor cells, and correlate with tumor burden in a variety of human solid malignancies at baseline and following antitumor treatment, thereby providing a useful platform to evaluate responses to novel antiangiogenic and immunotherapeutic agents.

endothelial cells, malignant cell proliferation, and migration, as well as indirect effects attracting host immune system cells such as macrophages and neutrophils. Myeloid cells in tumors produce secondary mediators that further promote tumor angiogenesis and growth (10,11). IL8 exerts its functions through the CXCR1 and CXCR2 receptors. The absence of IL8 in the mouse genome has hampered progress due to the fact that the mouse orthologs are not entirely equivalent, even though human IL8 is at least partially functional on the corresponding CXCR1 and CXCR2 mouse receptors (12). Therefore, the precise function of IL8 is difficult to address with experiments in search of genetic evidence.

High IL8 concentrations have been previously detected in serum or tissue specimens from patients with cancer and correlated with tumor size, depth of infiltration, stage, and prognosis. Reports in melanoma (13–15), non–small-cell lung cancer (NSCLC; refs. 16,17), prostate cancer (18), esophageal squamous cell carcinoma (19), soft sarcoma (20) and bone sarcoma (21), renal cell carcinoma (RCC; ref. 22), urothelial tumors (23,24), hepatocellular carcinoma (HCC; refs. 25,26), colorectal cancer (27), gastric cancer (28), pancreatic cancer (29), ovarian cancer (30), breast cancer (31), neurologic tumors (32), lymphoma (33,34), and others (5,35) are available, but its value as a cancer biomarker to predict changes in tumor burden and to monitor response to cancer treatment has been poorly explored.

All in all, preclinical and clinical data suggested that IL8 could be a good candidate to accurately appraise the numbers of tumor cells producing this chemokine at any given time point. Because of a short serum half-life, serum concentrations ought to closely follow changes in IL8-producing cells. Therefore, we performed this study to assess the correlation of IL8 with tumor burden in various malignant diseases and its relation with tumor response following anticancer therapy. IL8 could become a useful tool to evaluate response to different antitumor treatments, particularly to kinase inhibitors and immunotherapy, in which

the value of tumor imaging is limited because of the complex biologic mechanisms of action involved in these treatment modalities.

Materials and Methods

Patients

One hundred and twenty-six patients with confirmed diagnosis of solid tumors were included in this study from January 2003 to March 2014.

Tissue origins were melanoma ($n = 27$), RCC ($n = 27$), NSCLC ($n = 24$), HCC ($n = 33$), prostate cancer ($n = 6$), bladder cancer ($n = 3$), and others ($n = 2$). Clinical features are summarized in Supplementary Table S1. The protocol for the study was approved by local ethics committee (reference 111/2010) and all patients signed written informed consent.

Tumor burden was measured in all patients by physical examination or imaging studies, according to modified Response Evaluation Criteria in Solid Tumors (RECIST 1.1), with the modification that we measured all lesions and not only RECIST-target lesions. Simultaneously (± 2 weeks), we collected blood or/and urine samples from each patient.

For NSCLC and HCC, we estimated tumor stage using the American Joint Committee on Cancer (AJCC) tumor–node–metastasis (TNM) 2007 edition (36), and the Barcelona Clinic Liver Cancer (BCLC) staging criteria (37), respectively.

To assess variations in IL8 levels following treatment, we prospectively collected sequential blood samples at baseline and at the moment of evaluation of response from patients with melanoma treated with BRAF inhibitors (iBRAF) and ipilimumab.

To assess variation in IL8 levels following cytoreductive surgery, we prospectively collected sequential blood samples at baseline and 5 to 7 days after surgery in 7 patients. Clinical features are summarized in Supplementary Table S2.

Sample collection and biochemical assays

Peripheral blood samples were obtained by venipuncture (10 mL; BD Vacutainer glass serum tube) and centrifuged ($1,000 \times g$, 15 minutes) to isolate serum that was stored at $-80°C$ in aliquots until analysis. No previous freeze–thaw cycles were performed before analysis. Freezers were monitored and activity recorded in a quality-controlled tissue bank that follows standard operation procedures.

Lactate dehydrogenase (LDH) was analyzed by a kinetic spectrophotometric method using a kit from Roche on a Modular Analytics P800 analyzer (Roche) with results converted to 30°C.

C-reactive protein (CRP) was measured with an immunoturbidimetric assay using reagents from Roche (Roche Diagnostics GmbH) on a Hitachi modular P autoanalyzer (Roche/Hitachi).

Serum levels of IL8 were measured by a commercial enzyme-linked immunosorbent assay (ELISA) that detects both the monomer and dimer form (Human IL8 ELISA set; BD Bioescience Pharmingen), according to the

manufacturer's instructions. Serum levels of IL6 were measured by commercial ELISA (Human IL-6 Set; BD Biosciences Pharmigen) according to the manufacturer's instructions. All samples were measured in duplicate. The detection cutoff levels of the assay were 3.1 and 4.6 pg/mL for IL8 and IL6, respectively. The coefficient of variation was always <15%.

Urine samples were collected, centrifuged ($1,000 \times g$, 15 minutes), supernatant aliquoted, and frozen at $-80°C$ until analysis. For analysis, samples were thawed on ice. No previous freeze–thaw cycles were performed before analysis. IL8 levels were measured in duplicate at multiple serial dilutions using an ELISA specified for urine analyses following the manufacturer's recommendations (Human IL-8 ELISA Kit, RayBio, RayBiorthech, Inc.).

The optimal cutoff point for LDH and CRP was established by adapting the kit specifications to our laboratory and that were LDH = 292 U/L and CRP = 0.3 mg/L, respectively. For serum cytokines, we used different cutoffs in different malignant pathologies, calculated as a median of all IL8 or IL6 values obtained in melanoma and RCC, and IL8 values obtained in HCC. The calculated cutoff values were as follows: melanoma, IL8 = 60 pg/mL; IL6 = 4.6 pg/mL; RCC, IL8 = 10 pg/mL, IL6 = 4.6 pg/mL; and HCC, IL8 = 40 pg/mL.

Cell lines and cell culture

The HT-29 human CCR cell line was obtained from the American Type Culture Collection. UMBY and ICNI human melanoma cell lines were derived at the clinical facility Erlangen from primary surgical samples and were used at early-culture passages. The Me275 human melanoma cell line was derived from a surgically excised melanoma and was a kind gift from Dr. Pedro Romero (Ludwig Institute for Cancer Research, Lausanne, Switzerland). The 786-O human RCC cell line was kindly provided by Dr Luis del Peso (CSIC-UAM, Madrid, Spain). The human lung cancer cell line A549 was obtained from the American Type Culture Collection (cat. no., CRL-1573). The human HCC cell line HUH-7 was kindly provided by Dr. Brechot (INSERM, Paris, France).

Tumor xenografted mice

$Rag2^{-/-}IL2R\gamma c^{-/-}$ mice were obtained from The Jackson Laboratory and bred at our institution. Animal experiments were conducted in accordance with Spanish laws and approval was obtained from the animal experimentation committee of the University of Navarra (Pamplona, Spain; reference 034/10 approval).

A total of 5×10^6 HT-29 cells, 2×10^6 HUH7 or A549 cells, or 1×10^6 ICNI or UMBY cells were injected subcutaneously into the flank of $Rag2^{-/-}IL2R\gamma c^{-/-}$ mice in 50 and 100 µL of PBS, respectively. Thereafter, we sequentially collected blood samples and measured the size of the tumor every 3 to 4 days. The blood samples were centrifuged (13,000 rpm, 10 minutes), supernatant aliquoted, and frozen at $-80°C$ until analysis. Serum levels of IL8 were measured as specified for human blood samples.

RT-PCR assays

Total cellular RNA was extracted from cells using Maxwell 16 simply RNA Cells Kit (Promega) according to the protocol provided by the manufacturer. First-strand cDNA was synthesized from 2 µg of total cellular RNA with M-MLV reverse transcriptase (Invitrogen) in the presence of RNase Inhibitor (Invitrogen). Real-time PCR was carried out with iQ SYBR green supermix in an iQ5 real-time PCR detection system (Bio-Rad). The specific primers used were as follows: IL8, forward primer 5'-CCAGGAAGAAACCACCGGA-3' and reverse primer 5'-GAAATCAGGAAGGCTGCCAAG-3'; and for β-actin, forward primer 5'-TCCCTGGAGAAGAGC-TAACGA-3' and reverse primer 5'-AGGAAGGAAGGCTG-GAAGAG-3'. Samples were analyzed in triplicate and data were normalized by comparison with β-actin as an internal control. The amount of each transcript was expressed according to the formula $2^{C_t^{(b-actin)} - \Delta C_t^{(IL8)}}$, where C_t is the cycle at which the fluorescence increases appreciably above background fluorescence.

IL8 mRNA assay in tumor biopsies

Fresh samples were fixed with formol 10% and immediately after, the tissues were embedded in paraffin blocks. After cooling, the blocks were cut with a microtome making sections from 5 to 10 µm, which were mounted on a microscope slide.

After deparaffination, the samples were rehydrated and stained with hematoxylin to discern tumor tissue from healthy tissue. Then, the slides were washed with distilled water and DEPC-treated water to inactivate RNAse enzymes and with Glicerol 10% in TAE 10×. At this point, the pathologist selected the tumor area of each sample and scraped it with the aid of a fine needle.

Total RNA was extracted with the RecoverAll Total Nucleic Acid Isolation Kit (Ambion) according to the manufacturer's protocol. The concentration was quantified with a NanoDrop Spectrophotometer (NanoDrop Technologies). First-strand cDNA was synthesized from 0.5 µg of total cellular RNA with M-MLV reverse transcriptase (Invitrogen) in the presence of RNase inhibitor (Invitrogen). Real-time PCR assay was performed as described above.

Statistical analysis

The nonparametric Mann–Whitney U tests were applied to compare the concentrations of the tumor markers. Wilcoxon tests were used to compare changes in serum levels of tumor markers during treatment. Response and progressive disease were defined according to the RECIST 1.1. Best response was defined as the best objective response (stable disease, partial response, complete response, or progressive disease) assessed between the first day of treatment to progression, death, or last follow-up. Overall survival (OS) Kaplan–Meier curves were appraised from treatment initiation or IL8 determination to death or last date of follow-up and compared by the log-rank test. A two-tailed P value ≤ 0.05 was considered to be statistically significant. Statistical analyses were performed with SPSS 20.0 software (SPSS Inc.).

Results

IL8 quantitatively reflects the number of tumor cells

We determined concentrations of IL8 in culture supernatants of 786-0, HT29, HUH7, A549, and Me275 cancer cells lines. Figure 1A represents IL8 levels in relation to the absolute number of tumor cells seeded to culture wells 48 hours before harvesting the culture media. Importantly, quantitative data were consistent in all cell lines derived from different tissue origins, because 786-0 represents RCC, HT29 colorectal cancer, HUH7 HCC, A549 NSCLC, and Me275 melanoma. The level of production seems to be an inherent feature of each tumor cell line, because repeated experiments rendered very stable results. However, the lack of linearity in the curves shows that other variables are determining the final outcome. The output of IL8 into the supernatant is known to change in response to hypoxia.

We confirmed that the mRNA encoding for IL8 increased in colorectal cancer (HT29) and melanoma (UMBY and ICNI) cell lines under hypoxia (1% O_2) as shown in Supplementary Fig. S1A.

To further substantiate the potential of IL8 as a biomarker, we xenografted Rag2$^{-/-}$IL2Rγc$^{-/-}$ mice with HT-29 tumor cells and monitored over time both tumor size and serum IL8 concentrations. Figure 1B shows that IL8 serum concentrations increased proportionally to subcutaneous tumor volumes. Tumors were surgically removed on day 18 or 26, and as early as 2 hours, after surgery blood samples were drawn. As seen in Fig. 1B, serum levels of IL8 that had been increasing during tumor progression abruptly dropped to undetectable levels within 2 hours. These xenograft experiments were also performed with UMBY and ICNI melanoma cell lines (Fig. 1B) and with HUH7 (HCC)
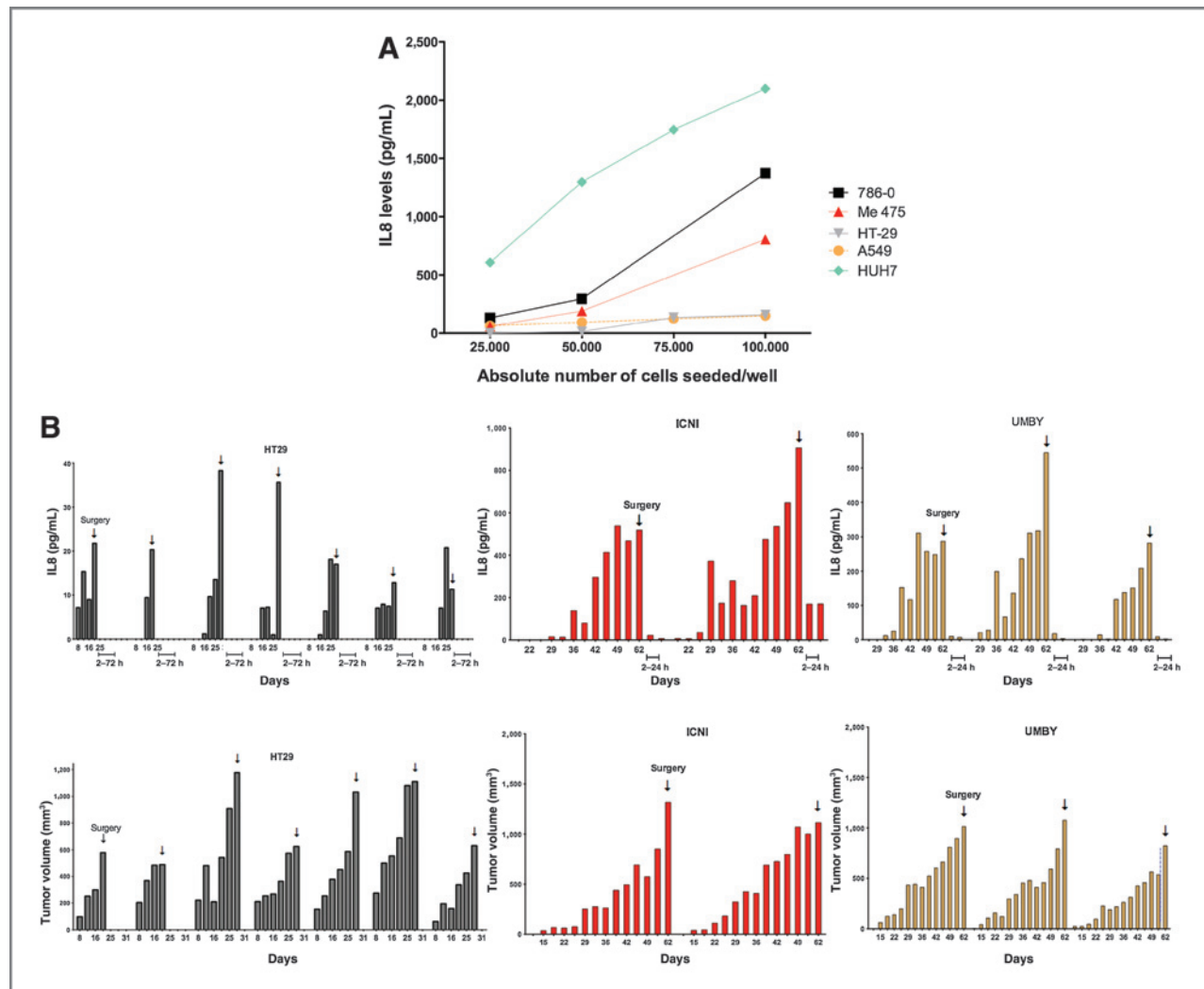
Figure 1. IL8 production by cultured and xenografted tumor cells reflects tumor cell number. A, concentrations of IL8 in the tissue 48-hour culture supernatants of the cell lines 786-0 (representing RCC), Me475 (representing melanoma), and HT29 (representing colorectal cancer) seeded at the indicated density. B, time course of the serum concentrations of IL8 (top) and tumor volume (bottom) in Rag2$^{-/-}$IL2Rγc$^{-/-}$ mice xenografted with the indicated cell lines. Individual mice are represented and at the time point, indicated by the arrow, the tumor was surgically removed. Serum IL8 concentrations are depicted at 2 and 24 hours (ICNI and UMBI) or 2, 12, 24, and 72 hours (HT29) after surgery.

and A549 (NSCLC) cell lines (Supplementary Fig. S1B) that more abundantly expressed IL8. Serum IL8 levels were higher, but the drop following surgery was also rapid and dramatic, further suggesting the potential of IL8 to monitor tumor burden in a variety of malignancies.

Our results were interpreted in the sense that IL8 serum concentrations may also allow to follow-up tumor burden in humans. To explore in the real surgical practice scenario if serum IL8 decreased following surgery, we have assessed IL8 serum levels before (day −1) and 5 to 7 days after either curative or tumor-debulking surgery in a small series of patients (Supplementary Fig. S2 and Supplementary Table S2). Indeed, surgery induced a dramatic and statistically significant ($P = 0.01$) reduction of IL8 levels in all cases. Similar results have been observed in a series of patients with ovarian cancer following debulking surgery (Dr. Frances Balkwill; personal communication).

IL8 serum concentrations indicate tumor burden and extent of disease in human patients

Because IL8 expression is shared by multiple human tumors of very different histologic origins, we correlated IL8 levels with tumor burden and disease stage in serum samples from patients with cancer. To confirm that IL8 is produced by tumor cells in some of our patients, IL8 mRNA expression was determined in the biopsy of three melanoma, confirming detectable expression by the dissected malignant cells (Supplementary Fig. S3).

To categorize tumor burden, we used in 16 melanoma and 23 patients with RCC the median of cumulative tumor diameters (100 mm for patients with metastatic melanoma and 150 mm for patients with RCC) as the cutoff value to categorize high-tumor burden and low-tumor burden groups. As shown in Fig. 2A, IL8 concentrations were clearly higher in patients with high-tumor burden than in patients with low-tumor burden ($P < 0.001$ for metastatic melanoma and $P < 0.01$ for RCC). IL6 and CRP concentrations as biomarkers of inflammation were also quantitated in 14 melanoma and 19 RCC patients' serum samples, showing a less significant correlation than IL8 with burden of disease (IL8, $P < 0.0001$; IL6, $P < 0.01$; CRP, $P < 0.001$; Supplementary Fig. S4). Indeed, the sensitivity and specificity of IL8 to recognize patients with high-tumor burden was superior than that of IL6 and CRP (specificity: IL8, 77%; IL6, 66%; CRP, 33%; sensitivity: IL8, 100%; IL6, 60%; CRP, 100%).

Figure 2. IL8 serum concentrations reflect tumor burden and extension. A, serum IL8 concentrations in two series of prospectively banked samples from melanoma ($n = 16$) and RCC ($n = 23$) for which tumor burden was assessed by image studies and physical examination. High ($\geq$100 mm melanoma; $\geq$150 mm RCC) and low (<100 mm melanoma; <150 mm RCC) tumor burden patients were categorized showing different levels of IL8 (median IL8 high-tumor burden: melanoma, 412 pg/mL; RCC, 54 pg/mL and low-tumor burden: melanoma, 19.57pg/mL; RCC, 7.8 pg/mL). B, measurements as in A, in a series of serum samples from NSCLC ($n = 21$) and HCC ($n = 30$), in relation to the AJCC and BCLC staging classifications, respectively. The lines show the median value for each group and statistical comparisons were made with Mann–Whitney U tests. (ns, nonsignificant; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$).
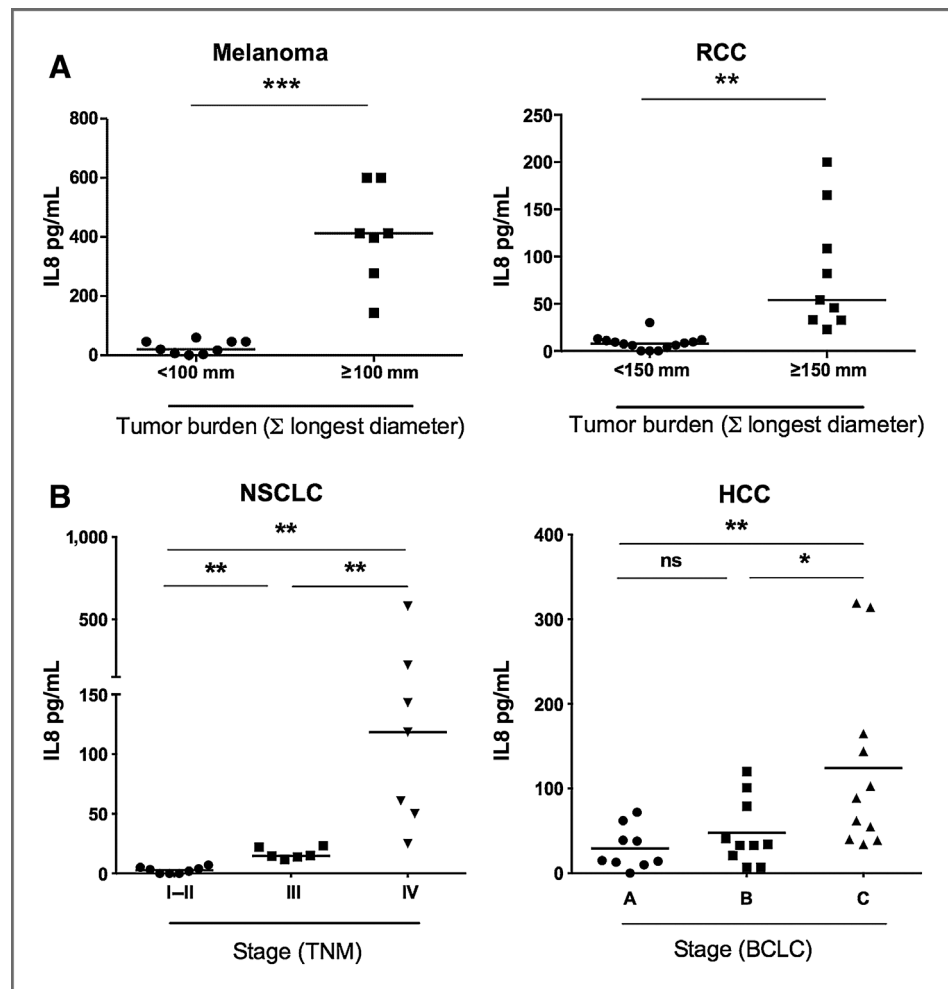
We also correlated IL8 levels with tumor stage in NSCLC ($n = 21$) and HCC ($n = 30$) patients. For NSCLC, IL8 was almost undetectable in stage I and II patients, while it increased gradually in stage III and IV patients ($P < 0.01$). As for HCC, IL8 concentrations were also higher in patients presenting more advanced disease ($P < 0.01$; Fig. 2B).

Objective responses to treatment can be assessed by monitoring IL8 serum concentrations

We assessed IL8 and LDH serum levels in 16 patients with melanoma treated with iBRAF before treatment, at best response and upon disease progression (Fig. 3A). Clinical characteristics are depicted in Supplementary Table S1. Levels of IL8 correlated with best clinical response ($P < 0.01$) and with progressive disease ($P < 0.05$). LDH levels

also correlated with best clinical response, although with a lower significance ($P < 0.05$), and did not correlate with progressive disease.

We also followed IL8 serum levels in 8 patients with metastatic melanoma treated in our institution with ipilimumab 3 or 10 mg/kg up to four doses. One patient presented a partial response, 3 patients had stable disease, and 4 progressed rapidly. IL8 serum values decreased in patients benefiting from ipilimumab, while they clearly increased in all the patients that progressed (Fig. 3B). OS correlated with variations in IL8 levels ($P = 0.035$; Fig. 3B, inset). This finding is not restricted to ipilimumab immunotherapy because in an ongoing series of patients with melanoma treated with anti-PD-1 mAbs, a similar correlation between IL8 levels and clinical response has been observed (data not shown).
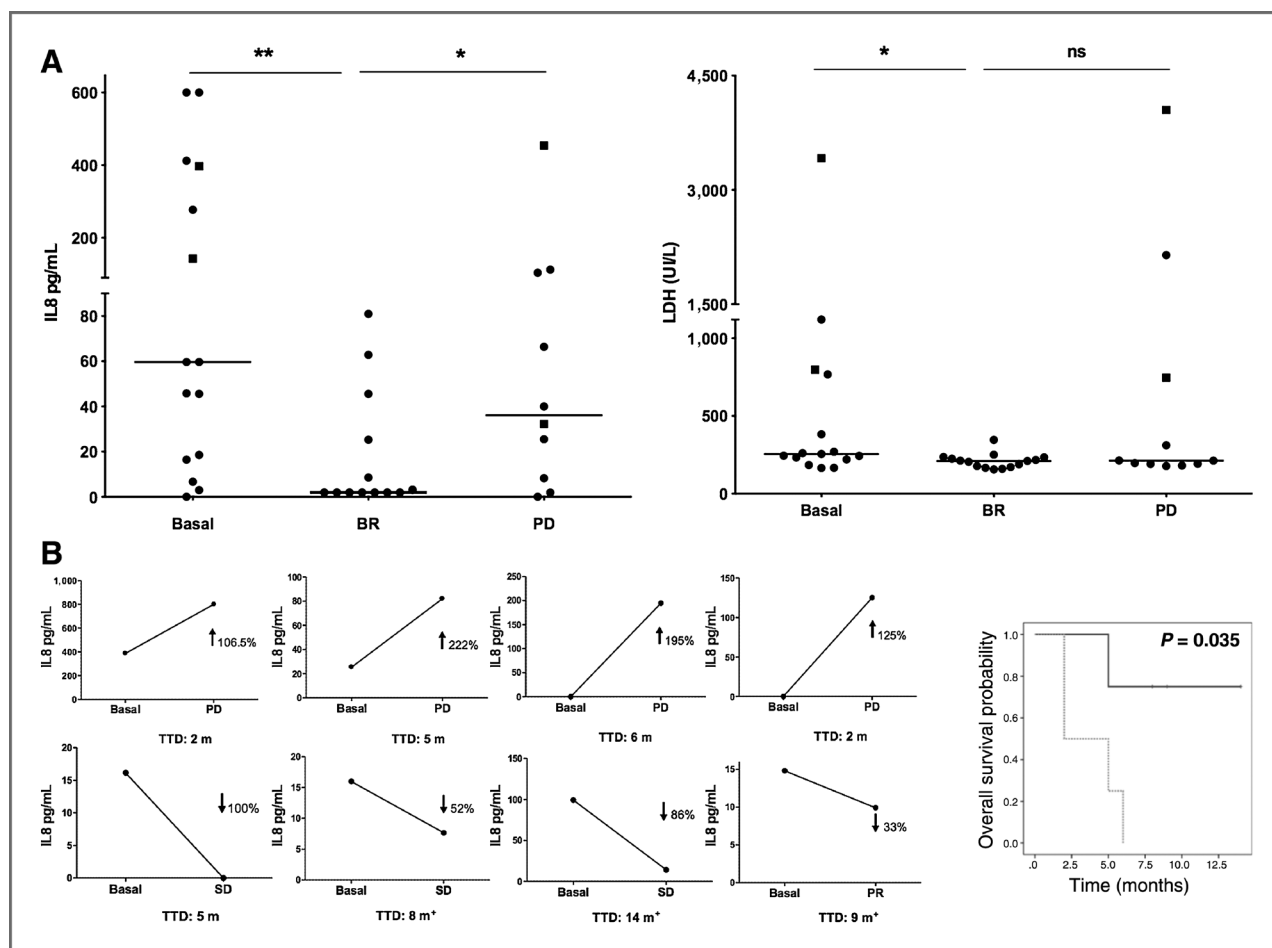
Figure 3. IL8 serum concentration reflects objective melanoma responses to iBRAF and an anti-CTLA4 mAb. A, serum concentrations of IL8 were measured in patients with melanoma ($n = 16$) treated with iBRAF (vemurafenib or dabrafenib) at baseline, at best response and at the time of progressive disease. The lines show the median value at each moment. Statistical comparisons were made using Wilcoxon tests (left). LDH levels in the same patients also reflect objective response (right). Squares represent nonresponding patients in which only progressive disease was observed, so their IL8 and LDH levels are therefore only represented at baseline and at the moment of progressive disease. BR, best response; PD, progressive disease. B, sequential follow-up IL8 serum in 8 patients with metastatic melanoma treated with ipilimumab (at 3 or 10 mg/kg). Top, progressors at baseline (before treatment) and upon clinical-radiologic evidence of progression (12–16 weeks after treatment onset). Bottom, 4 patients in whom stabilization of disease or partial response (as indicated in the corresponding graphs) were observed 12 to 16 weeks after treatment onset. At the bottom of each panel, the time to death (TTD) is indicated in months. Inset, a survival comparison of both groups (dotted line, patients with raised levels of IL8; continuous line, patients with decreased/stabilized levels of IL8). (ns, nonsignificant; *, $P < 0.05$; **, $P < 0.01$.)
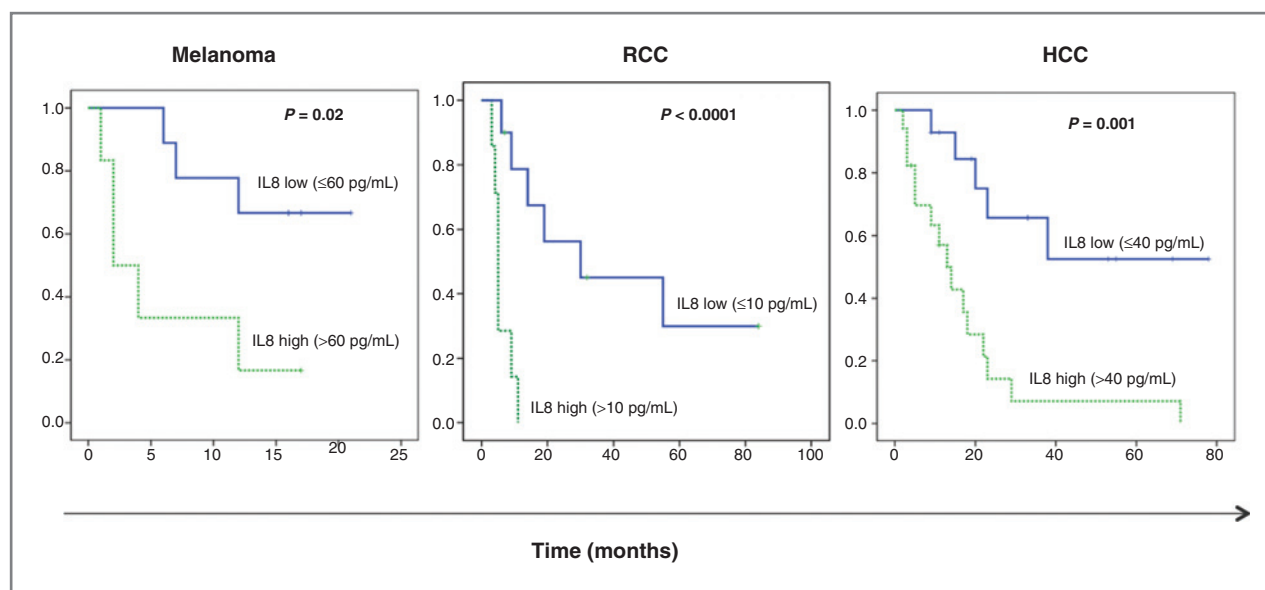
Figure 4. Serum IL8 predicts survival in a variety of malignant diseases. Kaplan–Meier plots representing survival for patients with melanoma ($n = 16$), RCC ($n = 23$) and HCC ($n = 33$) stratified by the median levels of IL8 for each tumor type. P values were calculated using the log-rank tests and are shown in each graph.

OS correlates with serum IL8 levels

We assessed the relation between the OS of melanoma ($n = 16$), RCC ($n = 23$), and HCC ($n = 33$) patients with IL8 serum levels. Clinical characteristics are described in Supplementary Table S1. We used the median concentration of IL8 in each tumor type to categorize patients with high or low IL8 levels (melanoma, 60 pg/mL; RCC, 10 pg/mL; HCC, 40 pg/mL).

As seen in Fig. 4, lower concentrations of IL8 in serum clearly correlated with longer survival in all tumor types [melanoma: hazard ratio (HR), 0.21; 95% confidence interval (CI), 0.05–0.92; $P = 0.038$; RCC: HR, 0.09; 95% CI, 0.01–0.4; $P = 0.004$; HCC: HR, 0.2; 95% CI, 0.07–0.56; $P = 0.02$].

Urine IL8 concentrations can be useful to monitor tumor burden in genitourinary malignancies

We hypothesized that urine IL8 levels could be used as a surrogate of blood levels. To study this possibility, we analyzed in parallel the serum and urine concentrations of IL8 in healthy volunteers, in patients with solid tumors, and in patients with urinary tract tumors that were in contact with the urinary tract, as assessed by CT scans.

Figure 5 represents IL8 concentrations in serum and urine. We divided the urine concentration by creatinine levels to correct for diuresis. The magnitude of tumor burden is shown between serum and urine concentration graphs. Healthy volunteers ($n = 12$) had either undetectable or very low IL8 concentrations in blood and urine. Eighteen patients with cancer were studied. Clinical characteristics are summarized in Supplementary Table S1. Twelve patients with tumors that did not involve the urinary tract presented increased serum IL8 levels that correlated with tumor burden (Fig. 5, top inset), but did not present high levels of IL8

in urine. Accordingly, the correlation of serum and urine IL8 concentrations was poor (Fig. 5, bottom inset). However, patients with tumors that were in contact with the urinary tract ($n = 6$), presented very high concentrations of IL8 in urine.

Discussion

IL8 is a polypeptide produced by malignant cells of different histologic origins (1). Reasons for deregulated IL8 biosynthesis in tumor cells are multiple and involve oncogenic transcriptional aberrations (5) and hypoxia (38).

We showed that in tumor cell cultures, the number of tumor cells correlates directly with the recovery of soluble IL8 in culture supernatants. Moreover, in melanoma and colorectal cancer xenografts, IL8 concentrations precisely correlated with tumor burden. This supports the hypothesis that IL8 output is a relatively constant parameter for a single tumor cell and that IL8 serum levels may accurately reflect the amount of tumor cells. Moreover, when tumor xenografts were excised, serum concentration of IL8 dropped very rapidly, becoming undetectable in a matter of hours. This likely reflects the rapid renal clearance of IL8, a consequence of its low-molecular weight, which is under the filtration threshold (39). Finally, IL8 significantly decreased in patients with cancer 5 to 7 days after disease-reduction surgery, confirming in patients the results observed in xenografted mice. All in all, our results are in line with previous reports in which stage or tumor load were correlated with serum IL8 concentrations (18,19,21,28,31).

None of the healthy volunteers that we studied showed increased IL8 in serum or urine. However, serum IL8 is not only increased in malignancies, because it is also an important acute inflammatory mediator that can increase upon
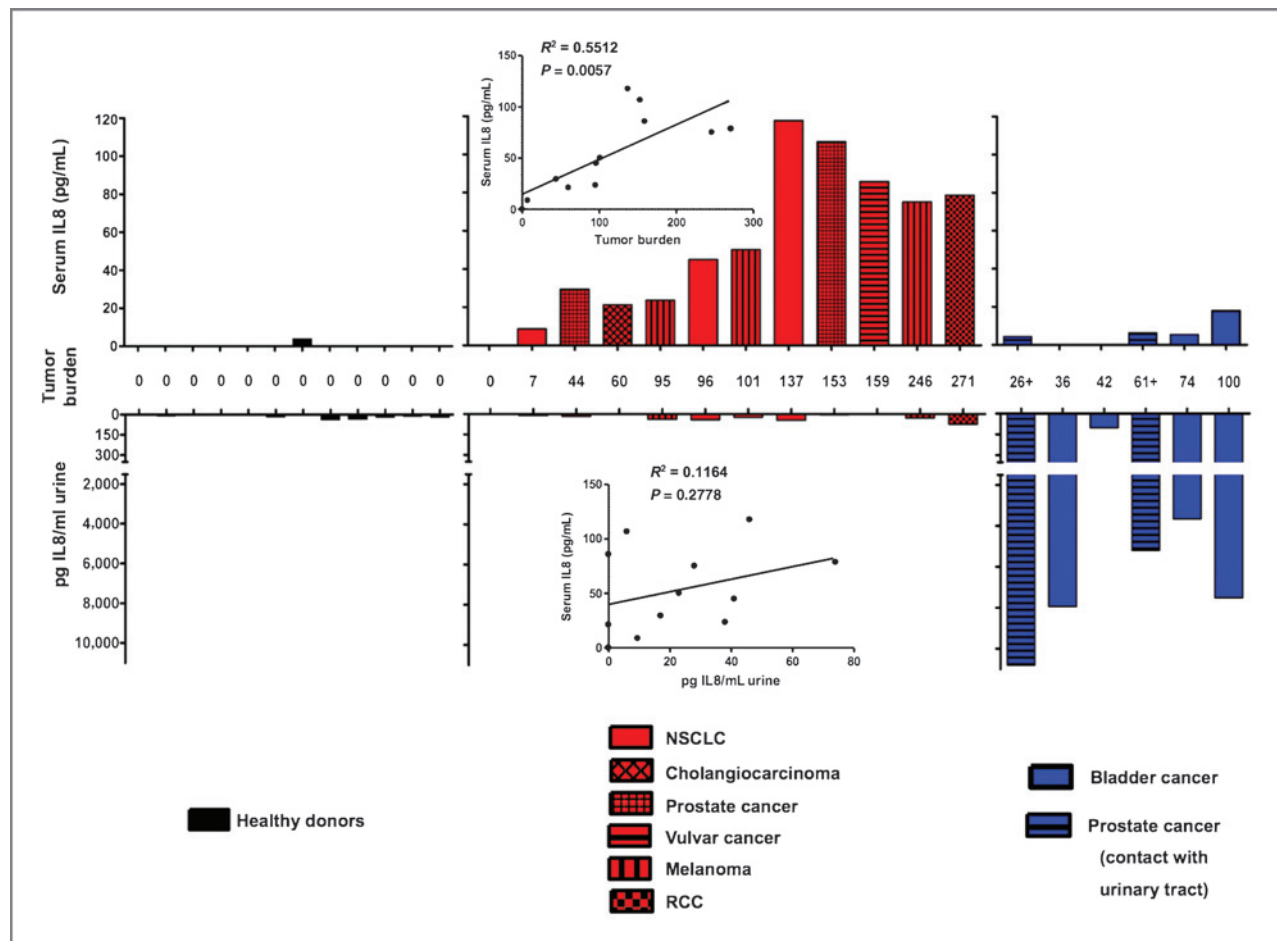
Figure 5. Serum and urine IL8 in patients with solid cancer, healthy volunteers, and urogenital tumors. Serum (top) and urine (bottom) IL8 concentrations were assessed in healthy donors ($n = 12$) and patients with cancer ($n = 18$) suffering carcinomas of the indicated color-coded histologic origins. Numbers between graphs depict tumor size as the sum of longest lesion diameters of each individual. Patients represented in the right panels were CT scan documented to host tumors in contact with the urinary tract (bladder or urethra) at the time of sample collection. Top inset, relation between tumor burden magnitude and serum IL8 levels. Bottom inset, the relation between urine and serum IL8 levels in patients with cancer.

infection or tissue damage (40), thus limiting its value as a biomarker to diagnose the presence of cancer. Therefore, we explored its potential to monitor the evolution of patients with cancer.

In our study, we made three important observations: (i) IL8 serum levels correlate with tumor burden in several tumor types; (ii) IL8 serum levels decrease or increase, respectively, following changes in tumor burden upon clinical responses or disease progressions induced by novel treatments, such as iBRAF or ipilimumab, and such variations correlate with changes in tumor burden; and (iii) IL8 concentrations in urine are increased in tumors that are in contact with the urinary tract, which we interpret as due to a direct output of IL8.

Regarding the value of serum IL8 to estimate tumor burden, we observed that there is a correlation between IL8 serum levels and tumor size and cancer stage in patients with melanoma, RCC, NSCLC, and HCC. These tumors lack adequate biomarkers that can be used in clinical practice, with the sole exception of α-fetoprotein (AFP) for a fraction

of cases of HCC (41). Therefore, the development of potential biomarkers for these tumors is an urgent unmet need. We also observed that patients with higher IL8 levels presented decreased survival, in accordance with previous reports (25–27,29,31,42–44). Although this fact may be explained by tumor burden, a contribution by a direct pathogenic role of IL8 cannot be excluded, because IL8 is involved in tumor vascularization (5,45) and chemotactically disorients migration of immune cells (12). Guida and colleagues (46) have reported that IL8 was unrelated with prognoses in patients with metastatic melanoma, even though the group with the highest levels of IL8 presented the lowest median OS. Other groups have reported lack of association of IL8 and prognosis (34,47–49). Complexity of IL8 functions, technical issues, and/or characteristics of the selected patient populations may explain such discrepancies. Therefore, the correlation between serum IL8 and prognosis deserves further investigation.

To assess response to therapy, a biomarker needs to reflect the amount of cancer cells in the organism. This could be

useful to evaluate responses without the need to perform imaging studies, or to interpret the occurrence of the "pseudo-progressions" that are observed with immunomodulatory antibodies (50). We observed that IL8 serum levels decreased and increased, respectively, in patients who presented objective responses or progressive disease following treatment with iBRAF or ipilimumab. This is concordant with three previous reports in which decrease of IL8 levels during treatment showed association with improved response to classical chemotherapy and radiotherapy (51–53). In the case of iBRAF, it must be borne in mind that IL8 is upregulated by the MAPK pathway, and hence decreases in IL8 may also reflect the pharmacodynamic effects of iBRAF on the control of IL8 transcription (54). Nonetheless, when patients eventually progress, IL8 levels increase despite continuous therapy with iBRAF. In addition, our data in patients with melanoma treated with ipilimumab and with anti-PD-1–blocking mAbs (Melero and colleagues; Unpublished Data), which are not supposed to directly affect IL8 transcription provide evidence for IL8 as an independent biomarker that monitors the burden of tumor cells, and thus can be used to monitor the progression of the disease and the response to therapy. In that way, IL8 showed a better correlation with tumor burden in the progression of the disease in patients with melanoma than LDH, the classical marker in this disease.

Urine determinations are more convenient for patients and health care providers. Previous reports have shown that IL8 is increased in the urine of patients with bladder cancer (55–57). Our data suggest that urine IL8 concentrations warrant further investigation as a biomarker in tumors that are in direct contact with the urinary tract, including bladder and prostate cancer. However, according to our results, urine IL8 does not seem to be a good marker of tumor burden in tumors that are not in contact with the urinary tract.

In conclusion, our study provides clear evidence that serum IL8 levels correlate with tumor burden and prognosis in patients with different tumor types, and that, because IL8 serum concentrations rapidly reflect changes in bioproduction by cancer cells, IL8 may become a helpful biomarker to monitor the clinical activity of novel cancer therapies, such as iBRAF or immunomodulatory mAbs.

Authors' Contributions
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