Serum IGF-I, IGFBP-3, and matrix metalloproteinase-7 levels and acquired chemo-resistance in advanced colorectal cancer

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Abstract

Insulin-like growth factor-I (IGF-I) is thought to have antiapoptotic and mitogenic properties in colorectal cancer, whereas IGF-binding protein-3 (IGFBP-3) seems to exert a pro-apoptotic effect. Additionally, matrix metalloproteinase-7 (MMP-7), an enzyme with *in vitro* ability to degrade IGFBP-3, has been shown to be a prognostic factor in advanced colorectal cancer (ACRC). We studied whether chemotherapy treatment for ACRC modulates IGF-I, IGFBP-3, and MMP-7 serum levels. In 41 patients undergoing first-line therapy for ACRC, serum levels of IGF-I, IGFBP-3, and MMP-7 were measured with immunoassays at baseline and every 3 months until progressive disease, or a maximum of five determinations, during a chemotherapy regimen of either FOLFOX or FOLFIRI therapies. Associations were assessed for paired samples, using *t*-test or Wilcoxon ranks test depending on normality of the variable, verified with Shapiro-Wilk test. An average of four extractions (range 3–5) were done, for a total of 157 determinations. Mean pretreatment values of IGF-I, IGFBP-3, and MMP-7 were 83 (95% CI, 73–92) ng/ml, 2372 (95% CI, 2121–2623) ng/ml, and 10.6 (95% CI, 7.21–13.98) ng/ml respectively. No significant changes in IGF-I were found, but a significant increase in IGFBP-3 serum concentrations was observed during or after chemotherapy treatment without progressive disease, compared with basal levels ($P<0.001$). A significant decrease in IGFBP-3 to 1983 ng/ml (95% CI, 1675–2292) and a significant increase in MMP-7 levels to 14.6 (7.6–21.7) ng/ml were observed at progression of disease compared with baseline and treatment levels ($P<0.001$). This study shows that IGFBP-3 and MMP-7 serum levels change during chemotherapy treatment. The increased MMP-7 levels at disease progression support the hypothesis that this protease could play a role in acquired resistance by degrading IGFBP-3.

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Introduction

The insulin-like growth factor (IGF) system is composed of two peptide ligands (IGF-I and IGF-II) that are mitogenic in normal and neoplastic cells, two cell surface tyrosine kinase receptors (IGF-IR and IGF-IIR), and a family of six IGF-binding proteins (IGFBPs 1–6). IGF-IR is activated by binding either to IGF-I or to IGF-II, binding the former with higher affinity than the latter. IGF-I and IGF-II act as growth factors by autocrine, paracrine, and endocrine mechanisms (Jones & Clemmons 1995, Davies *et al*. 2006).

Most circulating IGFs are produced in the liver and are subject to complex regulation by the GH and nutritional factors. IGFBPs modulate IGF-I and IGF-II bioavailability in both circulation and cellular micro-environment. IGFBP-3 binds to more than 95% of the IGF-I in serum and influences cell proliferation by

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modulating the access of IGFs to the IGF-IR (Pommier *et al*. 1993, Michell *et al*. 1997, Pollak *et al*. 2004, Davies *et al*. 2006).

IGFBP-3 acts as a regulator in the dividing and differentiation processes at the intestinal crypt. Normal colonic epithelium shows IGFBP-3 staining, especially of the cells in the top half of the crypt. On the contrary, undifferentiated colorectal cancer shows weak staining for IGFBP-3 (Williams *et al*. 2000). In fact, it seems that only the stromal component of the tumors, but not the malignant epithelial cells, expresses IGFBP-3 as a feedback mechanism of IGF-IR regulation (Jenkins *et al*. 2005). This could be related either to an aberrant promoter methylation of IGFBP-3 (Tomii *et al*. 2007) or to the mutational status of p53 (Buckbinder *et al*. 1995).

Metalloproteinases (MMPs) are a family of proteolytic enzymes that participate in the degradation of different components of the extracellular matrix (ECM) and in regulating the tumor microenvironment. MMP-7 (matrilysin) is the smallest of the more than 20 members of the group. While other MMPs are produced in the stroma, MMP-7 is synthesized in tumor cells of epithelial and glandular epithelial origin; it participates directly in the process of invasion and metastases in colorectal cancer (Zeng *et al*. 2002, Curran *et al*. 2004, Kurokawa *et al*. 2005). MMP-7 serum levels have also been associated with poor prognosis in metastatic colorectal cancer (Maurel *et al*. 2007).

Recent data have shown that MMP-7 possesses pan-IGFBP protease activity (Nakamura *et al*. 2005). MMP-7 proteolysis of IGFBP-3 plays a crucial role in regulating IGF-I bioavailability and thus promotes cell survival (Miyamoto *et al*. 2004). MMP-7 also participates in epithelial–mesenchimal signaling. MMP-7 cleavage of IGFBP-5 releases IGF-II that plays a role as an autocrine myofibroblast growth factor (Hemers *et al*. 2005).

In this study, we examined whether chemotherapy treatment in advanced colorectal cancer (ACRC) modulates IGF-I, IGFBP-3, and MMP-7 serum levels. In addition, we examined the relationship between IGF-I, IGFBP-3, and MMP-7 levels before treatment and at disease progression.

Methods

Study population

Patients were recruited from the metropolitan area served by our hospital in Barcelona. Between September 2001 and December 2004, a total of 120 chemonaive patients with ACRC undergoing first-line chemotherapy were consecutively enrolled for biological studies. Out of the 120 recruited patients, 41 individuals with a minimum of three serial determinations were selected for the study. CT scans were done every 3 months until disease progression. Data were collected prospectively. Written informed consent was obtained from every patient and the local Ethics Committee approved the study.

Measurements of IGF-I, IGFBP-3, and MMP-7

Serum samples were stored at −80 °C until analyzed. IGF-I, IGFBP-3, and MMP-7 levels were measured with immunoassays at baseline and then every 3 months until disease progression, or a maximum of five determinations, during a chemotherapy regimen of either FOLFOX or FOLFIRI therapies. MMP-7 (Quantikine, USA) was determined using a quantitative solid-phase sandwich enzyme-linked immunosorbent assay (ELISA) and tested in duplicate. MMP-7 technique can detect both pro- and active forms of recombinant human MMP-7.

Statistical analysis

The study was designed as a prospective observational exploratory study. Recorded variables were age, sex, date of birth, date of disease progression, death or last follow-up, performance status (Eastern Cooperative Oncology Group), number and site of metastasis, and serum levels of carcinoembrionary antigen (CEA), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), IGF-I, IGFBP-3, and MMP-7 as described above. Continuous variables are described as mean (95% CI) or median (range) depending on the fulfillment of normality criteria. Associations for paired samples were assessed using *t*-test or Wilcoxon ranks test depending on the normality of the variable, verified with the Shapiro–Wilk test.

Results

IGF-I and IGFBP-3 were measured in serum samples of 41 patients receiving FOLFOX or FOLFIRI as first-line therapy for ACRC. Median age was 68 (range 38–81) years and 27 patients (66%) were male. Six patients (14.6%) had received prior adjuvant chemotherapy (Table 1) and 27 patients (66%) had serum determination analysis at disease progression. Prior to chemotherapy, the mean serum concentrations of IGF-I and IGFBP-3 were 82.7 ng/ml (95%CI, 73.2–92.2) and 2372 ng/ml (95%CI, 2121–2623) respectively. We examined the associations of pretreatment serum

levels of IGF-I and IGFBP-3 with clinical and biological characteristics including age, gender, performance status, LDH, ALP, creatinine, liver involvement, glucose, and the number of organs affected. No statistically significant differences were found between IGF-I and IGFBP-3 and any of the clinical–biological variables studied. MMP-7 was higher in patients with liver involvement (median 7.7, range 3.4–55.8 ng/ml) than in those with an unaffected liver (median 5, range 3.4–9.6 ng/ml; $P=0.003$). MMP-7 was also higher in patients with LDH levels above 450 (median 7.8, range 5.2–42.3 ng/ml) than those with lower LDH levels (median 6, range 3.4–55.8 ng/ml; $P=0.043$).

Table 1 Clinical characteristics of patients at baseline

	Number of patients	%
Age, years		
Median	68	
Range	38–81	
Sex		
Male	27	65.9
Female	14	34.1
ECOG performance status		
0	19	46.3
1	15	36.6
2	7	17.1
Number of organs involved		
1	28	68.3
>1	13	31.7
Type of organs involved		
Liver	33	80.5
Other than liver	8	19.5
Previous adjuvant chemotherapy		
No	35	85.4
Yes	6	14.6
Serum CEA (ng/ml)		
Median	35	
Range	1.5–856	
Serum LDH (UI/l)		
Median	393.5	
Range	207–15 864	
Serum ALP (UI/l)		
Median	319	
Range	133–1825	
Serum basal MMP-7 (ng/ml)		
Median	6.9	
Range	3.4–55.8	
Serum basal IGF (ng/ml)		
Mean	82.7	
Standard deviation	30	
Serum basal IGFBP-3 (ng/ml)		
Mean	2372	
Standard deviation	785.9	

ALP, alkaline phosphatase; CEA, carcinoembrionary antigen; ECOG, Eastern Cooperative Oncology Group; IGF-I, IGF-I; IGFBP-3, IGF-binding protein-3; LDH, lactate dehydrogenase; MMP-7, Matrix metalloproteinase-7.

Analysis of IGF-I and IGFBP-3 levels for each patient showed a persistent and significant correlation before treatment ($r=0.5$, $P=0.001$), at 3 months while on chemotherapy ($r=0.44$, $P=0.004$), and at disease progression ($r=0.52$, $P=0.006$).

Table 2 shows the mean levels of IGF-I, IGFBP-3, and MMP-7 at baseline, before progression and at disease progression. During or after therapy but before disease progression, a significant increase in IGFBP-3 (mean 2527, 95% CI 2348–2707 ng/ml) was observed, compared with basal levels (mean 2372, 95% CI 2121–2623 ng/ml; $P<0.001$). In addition, a significant decrease in IGFBP-3 at disease progression was found (mean 1983 ng/ml (95% CI, 1675–2292). Using the IGF-I/IGFBP-3 ratio as a surrogate marker of IGF-I bioavailability, we found a decline in IGF-I/IGFBP-3 ratio during therapy and before progression (mean 0.033, 95% CI 0.030–0.036) compared with basal ratio (mean 0.037, 95% CI 0.033–0.041; $P<0.001$), rising again at progression (mean 0.047, 95% CI 0.026–0.067; $P<0.001$). Figure 1 depicts the changes of these biomarkers taking the baseline levels as the reference.

As shown in Table 2, mean MMP-7 levels increase at disease progression, 14.60 (95% CI 7.56–21.65) ng/ml compared with levels during therapy, 9.57 (95% CI 6.46–12.69) ng/ml ($P=0.001$). This correlates with decreased IGFBP-3 but not with an increase in IGF-1. Figure 2 plots the relationship between the oscillations observed for MMP-7 and IGFBP-3: the ratio of the level of each marker at progression over the mean of the previous measurements is plotted for each individual. On each axis, lines divide the plot by 1, distinguishing individuals who elevate the marker at progression (ratio >1) from those who do not (ratio ≤ 1). This figure depicts how IGFBP-3 decreases in most patients with an increase in MMP-7 at progression. Indeed, only one patient appears in the upper right quadrant and most of the patients are in the lower right quadrant.

Discussion

Our data show how, in previously untreated ACRC patients, IGF-I and IGFBP-3 serum levels oscillate during the evolution of the neoplastic disease. IGFBP-3 increases when the tumor is quiescent or responding to chemotherapy and falls at progression.

To our knowledge, our study is the first to examine the variation over time of IGF-I and IGFBP-3 levels and their association with response to therapy in ACRC. Basal serum levels of IGF-I and IGFBP-3 are in the range of previous studies exploring these

Table 2 Total plasma insulin-like growth factor-1 (IGF-I), IGFBP-3, IGF-1/IGFBP-3 ratio, and matrix metalloproteinase-7 (MMP-7; mean, 95% CI) at baseline, before progression, and at progression

	n	**IGF-I** (ng/ml)	**IGFBP-3** (ng/ml)	**IGF-1/IGFBP-3 ratio**	**MMP-7** (ng/ml)
At baseline	41	82.7 (73.2–92.2)	2372 (2120.7–2623.3)	0.037 (0.033–0.041)	10.60 (7.21–13.98)
Before progression[a]	89	79.6 (73.5–85.7)	2527.2 (2347.6–2706.9)†	0.033 (0.030–0.036)†	9.57 (6.46–12.69)
At progression	27	71.8 (60.6–83)*	1983.4 (1675.2–2291.7)*,‡	0.047 (0.026–0.067)‡	14.60 (7.56–21.65)‡

IGF-I, IGF-I; IGFBP-3, IGF-binding protein-3; MMP-7, matrix metalloproteinase-7. *$P<0.05$ compared with basal levels (corrected by Bonferroni). †$P<0.001$ compared with basal levels (corrected by Bonferroni). ‡$P<0.001$ compared with before progression levels (corrected by Bonferroni).
[a]'Before progression' values refer to a mean of values obtained every 3 months while undergoing therapy or after therapy but before progression.

biomarkers in a similar patient population (Miraki-Moud *et al*. 2001). Conflicting results have been reported in breast cancer patients treated with adjuvant chemotherapy. Regarding IGF-I, no changes or significant reductions were observed in three small studies with CMF or anthracycline-based schedules (Peyrat *et al*. 1998, Kajdaniuk & Marek 2000, Furstenberger *et al*. 2006). In a larger cohort of patients with breast cancer treated with dose-intensified adjuvant anthracycline and taxane therapy, IGF-I and IGFBP-3 levels increased significantly during therapy (Kummel *et al*. 2007). This last observation is supported by *in vitro* data showing that genotoxic drugs can increase IGFBP-3 protein levels in tumor cell lines (Zadeh & Binoux 1997, Grimberg *et al*. 2005, Patel *et al*. 2008). As described above, a significant decrease in IGFBP-3 was observed in our study at disease progression, compared with basal and treatment levels. In concordance with our findings, a study of patients diagnosed with advanced breast cancer and undergoing chemotherapy showed a decrease in IGF-I and IGFBP-3 during treatment; only the latter was a predictor of survival, mainly in patients with liver involvement (Holdaway *et al*. 2003).

A nested case-control study in the Physicians' Health Study (Ma *et al*. 1999) describes a correlation between IGF-I and IGFBP-3 ($r=0.64$) in patients diagnosed with colorectal cancer. These results are in line with the steady correlation found between these biomarkers in our study.

In our sample, we also observed that basal levels of serum IGF-I and IGFBP-3 were not associated to any of the biological variables that could affect progression-free survival and overall survival, supporting the idea that IGF-I and IGFBP-3 are probably more influenced by physiologically individual liver synthesis than by colorectal cancer status. In a previously published report, basal plasma levels of IGF-I and IGF-II, but not IGFBP-3, were associated to greater baseline symptomatology in patients with ACRC. By contrast, IGF-I, IGF-II, and IGFBP-3 were not associated to age, performance status, gender, or overall survival (Meyerhardt *et al*. 2005).

In addition, we found that the decrease in IGFBP-3 at disease progression was associated with increased MMP-7 levels. To our knowledge, increased MMP-7 level has not ever been described at post-chemotherapy disease progression, although increased proteolytic degradation of IGFBP-3 has been described in breast cancer patients (Helle *et al*. 2001). Furthermore, recent *in vitro* data have shown that MMP-7 degrades IGFBP-2 in colorectal cell lines via proteinase activity (Miyamoto *et al*. 2007).

The reasons for MMP-7 increases at disease progression after chemotherapy treatment are not well understood. We propose that hypoxia in pretreated tumors could increase over basal status. Under hypoxic conditions, one of the most highly induced genes is

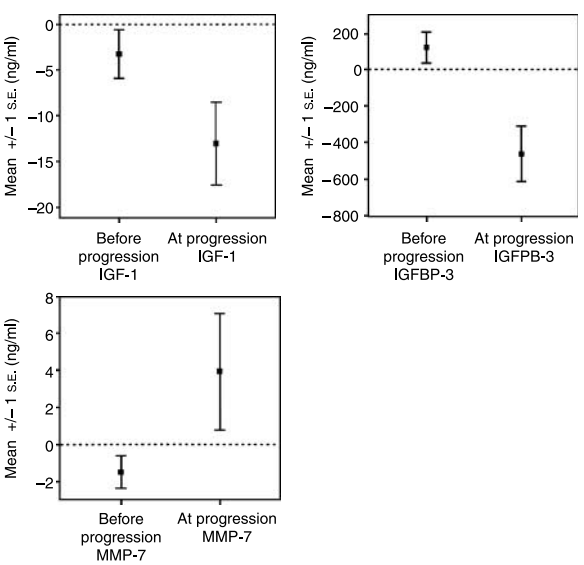

Figure 1 Mean changes to baseline of IGF-1, IGFBP-3, and MMP-7 before progression, and at progressive disease.

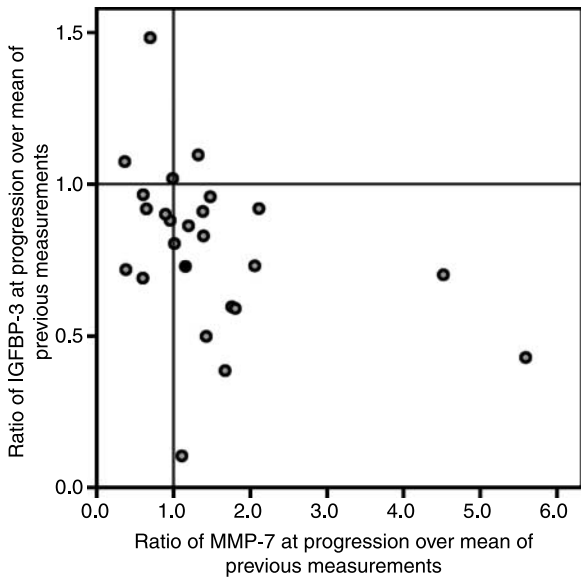

Figure 2 Correlation between levels of IGFBP-3 and MMP-7 before and during therapy compared with that at progressive disease (black dot denotes the overlapping of two individuals). IGFBP-3, IGF-binding protein-3; MMP-7, matrix metalloproteinase-7.

MMP-7 (Burke *et al*. 2003). Inhibition of MMP-7 prevented ERK1/2 activity and subsequent cell proliferation in response to hypoxia (Sabha *et al*. 2006). Therefore, we hypothesize that MMP-7 would, under hypoxic conditions, degrade IGFBP-3. As we have not observed increased IGF at disease progression, we cannot rule out decreased levels of IGFBP-3 as contributing to chemotherapy resistance by an IGF-IR-independent pathway. Evidence exists that a fragment of N-terminal (16 KDa) IGFBP-3 has pro-apoptotic activity, mediated through the suppression of the NF-kB survival pathway (Zadeh *et al*. 2006). It is also known that IGFBP-3 inhibits NF-kB activation in response to TRAIL-induced apoptosis (Williams *et al*. 2007).

Selection biases are probably irrelevant as long as the sample was collected in a prospective and correlative manner in a prespecified geographic population and time period. This should provide an adequate representation of the area served by the center. Absence of a control group should not be a concern for a descriptive study, as long as the data are analyzed in a paired manner until disease progression. These results, to our knowledge, are the first description of the evolution of these biomarkers during therapy and follow-up in patients with ACRC and allow us to generate the proposed hypothesis about their interaction and role in response to chemotherapy and in cancer progression. Further knowledge and prospective validation of these findings may lead to tools for predicting disease progression and individualizing therapies.

We can conclude that MMP-7 serum levels rise after chemotherapy, degrading IGFBP-3 and decreasing serum levels of this protein. The finding of increased MMP-7 levels at disease progression supports the hypothesis that this protease plays a role in acquired resistance by degrading IGFBP-3.

Declaration of interest

There is no conflict of interest.

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