Clinical Cancer Research

Aprataxin Tumor Levels Predict Response of Colorectal Cancer Patients to Irinotecan-based Treatment

Higinio Dopeso[1], Silvia Mateo-Lozano[1], Elena Elez[6], Stefania Landolfi[3], Francisco Javier Ramos Pascual[6], Javier Hernández-Losa[3], Rocco Mazzolini[1], Paulo Rodrigues[1], Sarah Bazzocco[1], Maria Josep Carreras[4], Eloy Espín[5,7], Manel Armengol[5,7], Andrew J. Wilson[8], John M. Mariadason[9], Santiago Ramon y Cajal[3], Josep Tabernero[6], Simo Schwartz, Jr.[2,7], and Diego Arango[1,7]

Abstract

Purpose: Irinotecan (CPT11) treatment significantly improves the survival of colorectal cancer patients and is routinely used for the treatment of these patients, alone or in combination with other agents. However, only 20% to 30% of patients show an objective response to irinotecan, and there is great need for new molecular markers capable of identifying the subset of patients who are unlikely to respond.

Experimental Design: Here we used microarray analysis of a panel of 30 colorectal cancer cell lines and immunohistochemistry to identify and validate a new biomarker of response to irinotecan.

Results: A good correlation was observed between irinotecan sensitivity and the expression of aprataxin (*APTX*), a histidine triad domain superfamily protein involved in DNA repair. Moreover, using an isogenic *in vitro* system deficient in *APTX*, we show that aprataxin directly regulates the cellular sensitivity to camptothecin, suggesting that it could be used to predict patient response to irinotecan. We constructed a tissue microarray containing duplicate tumor samples from 135 patients that received irinotecan for the treatment of advanced colorectal cancer. Immunohistochemical assessment of the tumor levels of aprataxin showed a significant association with treatment response and patient survival. Patients with low aprataxin had longer progression-free (9.2 versus 5.5 months; $P = 0.03$) and overall survival (36.7 versus 19.0 months; $P = 0.008$) than patients with high tumor aprataxin. No associations were found between coding *APTX* variants and aprataxin levels or camptothecin sensitivity.

Conclusions: These results show that aprataxin tumor levels can be used to identify patients with low probability of response to irinotecan-based therapy who are ideal candidates to receive treatment with alternative agents in an attempt to improve patient survival. *Clin Cancer Res; 16(8); 2375–82. ©2010 AACR.*

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/16/8/2375/1995421/2375.pdf by guest on 21 July 2024

Colorectal cancer accounts for one million new cases and >500,000 deaths worldwide every year, and the treatment options available are far from optimal (1). Curative treatment of these patients involves surgery and/or chemotherapy. For patients with advanced disease, palliative chemotherapy is often administered and can significantly improve their quality of life and overall survival. For over four decades the pyrimidine analogue 5-fluorouracil (5-FU) has been the gold standard for the treatment of colorectal cancer. However, only about 20% to 30% of colorectal cancer patients benefit from the treatment with 5-FU, either in the adjuvant setting or for the treatment of advanced disease (2, 3). More recently, additional chemotherapeutic agents have been approved for the treatment of these patients and are now in common use. These include the topoisomerase I inhibitor irinotecan (CPT11), the platinum compound oxaliplatin, and monoclonal antibodies against the epidermal growth factor receptor (EGFR) and the vascular endothelial growth factor (VEGF). These new agents have increased the percentage of advanced-disease patients with an objective response to approximately 50% and the overall survival to almost 24 months (4–8).

The lack of response of approximately half of the patients treated highlights the need for new chemotherapeutic agents. In addition, because the different chemotherapeutic agents currently available are effective only in overlapping subsets of patients, it would be very useful to have markers capable of discriminating the patients who are likely to

Authors' Affiliations: Groups of [1]Molecular Oncology and [2]Drug Delivery and Targeting, Molecular Biology and Biochemistry Research Center (CIBBIM-Nanomedicine), Departments of [3]Pathology, [4]Pharmacy, and [5]Surgery, Vall d'Hebron Hospital, and [6]Medical Oncology Department, Vall d'Hebron University Hospital, Barcelona, Spain; [7]CIBER de Bioingeniería, Biomateriales y Nanomedicina (CIBER-BBN), Zaragoza, Spain; [8]Department of Obstetrics and Gynecology, Vanderbilt University Medical Center, Nashville; and [9]Ludwig Institute for Cancer Research, Melbourne Centre for Clinical Sciences, Austin Health, Heidelberg, Victoria, Australia

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Corresponding Author: Diego Arango, Group of Molecular Oncology, Molecular Biology and Biochemistry Research Center, CIBBIM-Nanomedicine, Vall d'Hebron Hospital Research Institute, Passeig Vall d'Hebron 119-129, Barcelona 08035, Spain. Phone: 34-93-274-6739; Fax: 34-93-489-3893; E-mail: darango@ir.vhebron.net.

doi: 10.1158/1078-0432.CCR-09-3275

©2010 American Association for Cancer Research.

Translational Relevance

Irinotecan is commonly used for the treatment of advanced colorectal cancer. However, only 20% to 30% of these patients show an objective response to this topoisomerase I inhibitor and it is currently not possible to predict the probability of patient response. Here we show that aprataxin tumor levels can be used to separate two groups of colorectal cancer patients that show an overall survival difference of almost 1.5 years following irinotecan treatment. Aprataxin tumor levels can be used alone or in combination with other markers to make informed decisions regarding the type of treatment that is likely to be most effective for individual patients.

respond to each one of the available agents, in an attempt to improve the clinical management of these patients using a more personalized chemotherapeutic approach. A number of molecular markers capable of predicting the probability of response to these agents have been investigated over the last decades. For example, the tumor levels of the target of 5-FU, thymidylate synthase, the nucleotide excision repair gene excision repair cross-complementing 1 (ERCC1), or the mutational status of the oncogene *KRAS*, can predict the response to 5-FU, oxaliplatin, and cetuximab, respectively (9–11). However, there has been limited progress in the identification of markers capable of predicting response to irinotecan-based treatment.

Topoisomerase I (TOP1) regulates DNA supercoiling during replication by causing single-strand breaks and religation. Irinotecan is a camptothecin derivative that is converted to SN-38 by plasma and cellular carboxylesterases, and causes DNA damage by stabilizing a normally transient covalent complex between TOP1 and DNA, leading to DNA strand breaks, replication arrest, and apoptosis (12). Irinotecan has similar response rates to 5-FU plus leucovorin when used as a single agent for the treatment of advanced colorectal cancer (8), but combined treatment with these three agents (FOLFIRI) significantly improves response rates and survival of these patients (8, 13). Addition of the newer biological agents such as cetuximab and bevacizumab to the FOLFIRI regimen results in further improved response, and irinotecan is therefore commonly used for colorectal cancer treatment (6, 7).

Although high tumor levels of topoisomerase I have recently been reported to identify a small subgroup of patients with good response to irinotecan (14), these results were not confirmed in an independent study (15), and there is currently great need for additional markers of response to treatment with this agent. Using high-throughput techniques like microarray analysis, the levels of expression of thousands of genes can be rapidly investigated. Microarray analysis of cell lines that are thoroughly characterized for their sensitivity to chemotherapeutic agents *in vitro* has been shown to be a powerful approach to identify expression signatures associated with drug response. Moreover, these profiles of expression correlate with patient response to these agents (16–18). Using this approach, we have identified aprataxin as a candidate new marker of response to irinotecan treatment (18). Aprataxin (*APTX*) is a recently described gene (19, 20) that is involved in the repair of DNA strand breaks caused by various DNA damaging agents, including H_2O_2, methyl methane sulfonate, and the irinotecan-related compound camptothecin (21, 22). Germline mutations in *APTX* have been reported to cause ataxia-ocular apraxia 1, a neurologic disorder characterized by early cerebellar ataxia, oculomotor apraxia, early areflexia, and late peripheral neuropathy (19, 20). Here, we investigate the potential of assessing tumor levels of aprataxin as a maker capable of identifying a subset of advanced colorectal cancer patients with low probability of response to irinotecan-based treatment. Using immunohistochemical staining of tissue microarray sections containing duplicate samples of 135 primary tumors from colorectal tumors, we found that high levels of expression of aprataxin are associated with poor response to irinotecan-based chemotherapy.

Materials and Methods

Characterization of sensitivity to camptothecin. The sensitivity to camptothecin was characterized in a panel of 30 colorectal cancer cell lines: Caco-2, Colo201, Colo205, Colo320, Dld-1, HCT116, HCT-15, HCT-8, HT29, LoVo, LS174T, RKO, SK-CO-1, SW1116, SW403, SW48, SW480, SW620, SW837, SW948, T84, WiDr, HT29-Cl.16E, HT29-Cl.19A, LIM1215 and LIM2405, HCC2998, KM12, RW2982, and RW7213. All cell lines were obtained and grown as reported before (18), and tested for mycoplasma contaminations using the PCR Mycoplasma Detection Set (Takara Bio). The dose resulting in 50% reduction in the growth of the cell line was calculated as previously reported using the sulforhodamine B method (18, 23). Cells were exposed for 72 h to the following concentrations of camptothecin: 0.001, 0.005, 0.0075, 0.01, 0.025, 0.05, 0.1, 0.25, 0.5, 1, and 2 μmol/L. The induction of apoptosis after 72 h exposure to the indicated concentrations of camptothecin was also measured to evaluate drug sensitivity using propidium iodide staining and flow cytometry as previously reported (24, 25). All experiments were carried out at least three times in triplicate. The unique peaks for 2n and 4n DNA content in these assays indicated the absence of cross-contamination between lines. We also used the parental chicken B-lymphocyte cell line DT40, a subline where *APTX* was disrupted by homologous recombination, and a derivative line where *APTX* was reintroduced into the null cells (26).

RNA extraction and quantitative reverse transcriptase-PCR. Total RNA of colorectal cancer cell lines was extracted with the RNeasy Midi Kit (Qiagen) and reverse transcribed using the cDNA Archive Kit (Applied Biosystems). Aprataxin levels were analyzed using SYBR Green

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/16/8/2375/1995421/2375.pdf by guest on 21 July 2024

Master Mix (Applied Biosystems) and the primers APTX-F: AAGCTGGACTTGAACCCCTA; APTX-R: CTGTCCTGT-CTCACCAACCA. β-Actin was used as a standardization control: β-actin F: CACCTTCACCGTTCCAGTTT; β-actin R: GATGAGATTGGCATGGCTTT. Relative mRNA levels were assessed using the $2^{-\Delta\Delta Ct}$ method as described before (17, 25).

Patients. A total of 135 colorectal cancer patients with metastatic disease receiving irinotecan-based chemotherapy at Vall d'Hebron University Hospital (Barcelona, Spain) were included in the study. Table 1 summarizes the clinicopathologic information of the patients. Response to the chemotherapeutic treatment was evaluated by computed tomography using response evaluation criteria in solid tumors (RECIST) criteria (27). The median follow-up time of the patients in this study was 4.6 y. The study was carried out according to Human Investigations and Ethical Committee–approved research protocols.

Tissue microarrays and immunohistochemistry. After histologic examination of H&E-stained sections of formalin-fixed, paraffin-embedded tumor samples, areas containing a high proportion of tumor cells were selected from all 135 patients. Duplicate 1.2-mm cores of tumor samples from every patient were arrayed in a fresh paraffin block using a Beecher Instruments tissue arrayer (Beecher Instruments).

Unstained 4-μm sections from the tissue microarrays were mounted on slides coated with 3-aminopropyl-triethoxy-silane (Sigma). Sections were deparaffinized in xylene, and rehydrated through a graded alcohol series and distilled water. Antigen retrieval was achieved with preheated citrate buffer 10 mmol/L (pH 6) for 20 min to 95°C. For immunohistochemical analysis, the commercial Novolink Polymer Detection System (Novocastra Laboratories) was used according to the manufacturer's instructions. An aprataxin rabbit polyclonal antibody raised against the COOH-terminus of human aprataxin was used at a 1:100 dilution (4°C overnight; Aviva Systems Biology Corp). Staining was visualized using a 3,3-diaminobenzidine solution, and sections were counterstained with Mayer's hematoxylin, rinsed in water, dehydrated through a series of ethanol solutions, cleared in xylene, and mounted. Aprataxin levels were evaluated blinded from the clinical data. A semi-quantitative scale from 0 to 3 was used to measure the intensity of the staining. Absence of aprataxin immunostaining was scored as 0, and low, moderate, and high aprataxin levels were scored as 1, 2, and 3, respectively (Fig. 3A-D). The average score of duplicate samples was used in subsequent analyses. Aprataxin levels were considered low if average immunostaining score was <2 and high if it was ≥2.

Table 1. Clinical features of the 135 patients in the series used in this study

	Total	Low aprataxin	High aprataxin	*P*
Sex, no. (%)				
Female	50 (37.0)	20 (30.3)	30 (43.5)	0.15*
Male	85 (63.0)	46 (69.7)	39 (56.5)	
Age (y)	62.4	63.0	61.9	0.78†
Irinotecan regimen, no. (%)				
Irinotecan monotherapy	60 (46.9)	23 (37.1)	37 (56.1)	0.1‡
FOLFIRI	41 (32.0)	26 (41.9)	15 (22.7)	
FOLFIRI + bevacizumab	8 (6.3)	4 (6.5)	4 (6.1)	
FOLFIRI + cetuximab	19 (14.8)	9 (14.5)	10 (15.2)	
Degree of differentiation of tumor, no. (%)				
Good	13 (10.4)	7 (11.7)	6 (9.2)	0.44‡
Moderate	74 (59.2)	38 (63.3)	36 (55.4)	
Poor	38 (30.4)	15 (25)	23 (35.4)	
Objective response				
Complete response	9 (7.7)	7 (11.9)	2 (3.5)	0.13‡
Partial response	30 (25.9)	17 (28.8)	13 (22.8)	
Stable disease	29 (25)	16 (27.1)	13 (22.8)	
Progressive disease	48 (41.4)	19 (32.2)	29 (50.9)	
Median progression-free survival (mo)	6.7	9.2	5.5	0.03§
Median overall survival (mo)	26.3	36.7	19.0	0.008§

NOTE: *P* values calculated using Fisher's exact test, Mann-Whitney test, χ^2, or logrank test for the comparison of high and low Aprataxin. FOLFIRI: irinotecan with 5-FU and folinic acid.

*Calculated using Fisher's exact test.

†Calculated using Mann-Whitney test.

‡Calculated using χ^2.

§Calculated using Logrank test.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/16/8/2375/1995421/2375.pdf by guest on 21 July 2024

Transfections and Western blotting. pEGFP-C2 (BD Biosciences Clontech) or pEGFP-APTX (containing amino acids 2-342 of aprataxin and EGFP fused to the NH_2-terminus, a kind gift from Professor Martin F. Lavin; Queensland Institute of Medical Research, Radiation Biology and Oncology, Brisbane, Australia) were transfected into HEK293T cells using the calcium phosphate precipitate method as previously reported (28). Briefly, 5 μg of plasmid DNA were resuspended in a total volume of 86 μL of HBS (280 mmol/L NaCl, 50 mmol/L HEPES, 1 mmol/L Na_2HPO_4, pH 7.12, at 25°C) and 5 μL of a 2.5 mol/L $CaCl_2$ solution were added. This solution was pipetted dropwise to a 6-well plate containing 1.5×10^6 HEK293T cells seeded 16 h earlier. Cells were harvested 48 h posttransfection and extracts of total protein (100 μg) in radioimmunoprecipitation assay buffer were loaded on a 10% acrylamide gel. After gel electrophoresis, proteins were transferred to a nitrocellulose membrane and probed with an anti-aprataxin (1:400; Aviva Systems Biology) or anti-GAPDH (1:2,000; clone 6C5; Santa Cruz Biotechnology) antibody as previously described (29). GAPDH served as a loading control.

Sequencing of coding* APTX *polymorphisms. The possible association between known (National Center for Biotechnology Information database) polymorphisms in the coding sequence of *APTX* were investigated by direct sequencing of 10 colon cancer cell lines that vary in their sensitivity to camptothecin. Briefly, the corresponding genomic DNA fragments were PCR amplified (See Supplementary Table S1

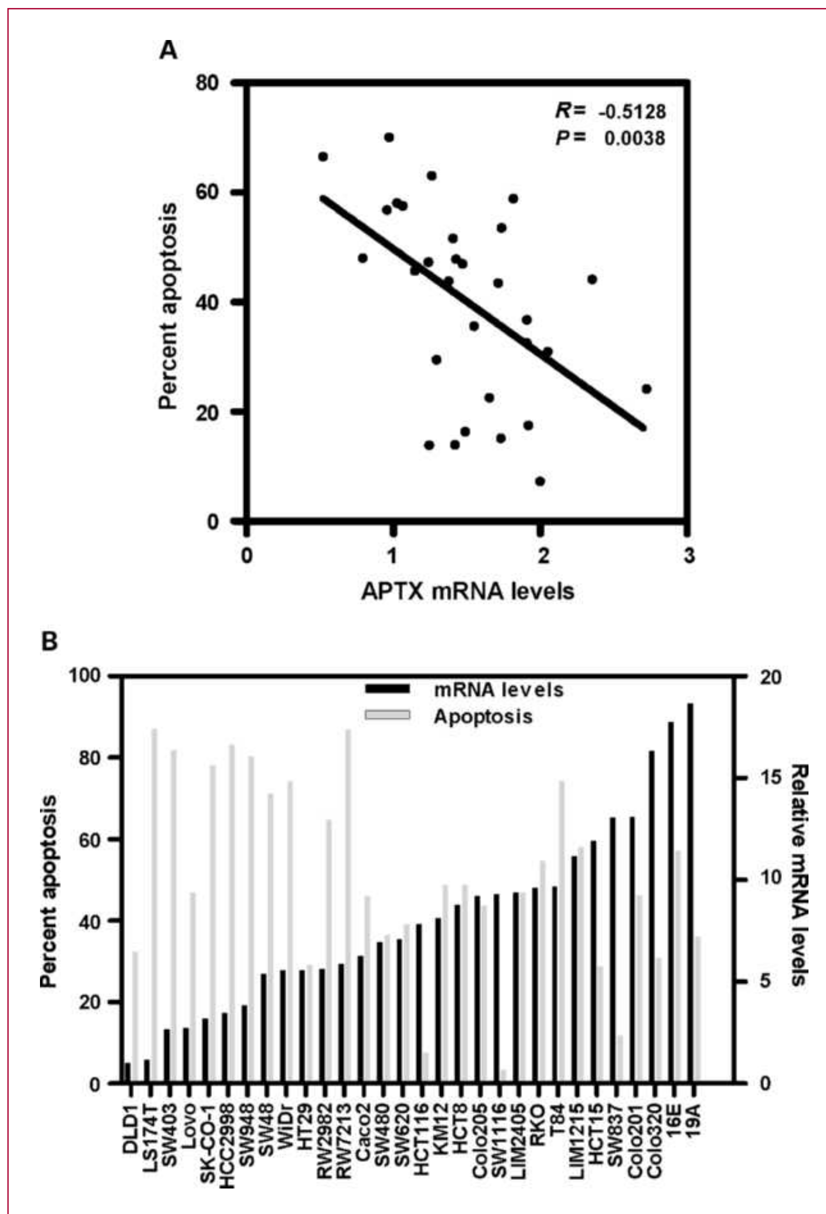

Fig. 1. Correlation between aprataxin (APTX) levels and camptothecin sensitivity. A, there was a negative correlation between mRNA expression levels in a panel of 30 colorectal cancer cell lines and the induction of apoptosis in response to treatment with 1 μmol/L camptothecin for 72 h. B, individual levels of aprataxin and the apoptotic response in these 30 cell lines.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/16/8/2375/1995421/2375.pdf by guest on 21 July 2024

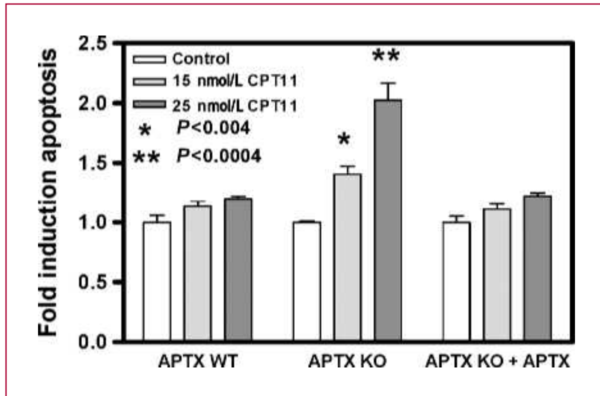

Fig. 2. *APTX* inactivation results in camptothecin sensitization. Exposure of parental DT40 cells (DT40 WT) to 15 and 25 nmol/L camptothecin for 72 h led to a modest induction of apoptosis. However, targeted inactivation of *APTX* (DT40 KO) resulted in a significantly increased induction of apoptosis in response to camptothecin treatment. Reintroduction of *APTX* into DT40 null cells (DT40 KO + APTX) completely restored the resistant phenotype of the parental DT40 cells.

for primer sequence) and ExoSap-purified. Sequencing reactions were carried out with BigDye Terminator Cycle Sequencing kit and sequenced using an ABI Prism 3100 sequencer (both from Applied Biosystems). Variations in the large deletion rs71731903 (805 bp) were studied by PCR amplification and agarose gel electrophoresis to assess size differences (see Supplementary Table S2).

Statistical analysis. Survival curves were constructed using the method of Kaplan and Meier and survival differences assessed using the Logrank test. The Cox proportional hazards model was used to assess the simultaneous contribution on overall survival of the following covariates: sex, age, histologic grade, and aprataxin tumor levels. Fisher's exact test, and χ^2 and Mann-Whitney test were used to assess differences between clinicopathologic parameters in patients with high and low levels of aprataxin (Table 1). *P* values <0.05 were considered to indicate statistical significance.

Results

Aprataxin levels inversely correlate with the sensitivity of colon cancer cell lines to camptothecin. We previously reported a profile of gene expression that is associated with the sensitivity of colon cancer cell lines to the irinotecan analog camptothecin. Microarray analysis of the levels of expression of >9,000 genes in a panel of 30 colorectal cancer cell lines identified a significant association between aprataxin expression levels and sensitivity to camptothecin (18). In good agreement, aprataxin has been recently shown to be involved in the repair of single-stranded DNA (22). Expression values obtained by microarray analysis were independently validated using quantitative real-time reverse transcriptase-PCR in these 30 colorectal cancer cell lines. A good correlation was observed between expression values obtained with both techniques (Pearson $R = 0.5$; $P = 0.006$). Moreover, aprataxin mRNA levels showed excellent correlation with both camptothecin-induced growth inhibition (GI_{50}; Pearson $R = 0.4$; $P = 0.03$) and induction of apoptosis (Fig. 1; 0.1 and 1 μmol/L; $R < -0.51$; $P < 0.004$). In addition, we show that camptothecin treatment does not affect the expression of aprataxin in colon cancer cells (Supplementary Fig. S1).

Aprataxin regulates the sensitivity to camptothecin. To directly assess the role of aprataxin on camptothecin sensitivity we used an engineered *in vitro* system where both alleles of *APTX* were targeted by homologous recombination. Figure 2 shows that treatment of parental DT40 cells with 15 to 25 nmol/L camptothecin for 72 hours resulted in a modest induction of apoptosis. However, targeted inactivation of *APTX* significantly ($P < 0.004$) sensitized cells to camptothecin treatment. Moreover, reintroduction of *APTX* into *APTX*-null cells completely restored the camptothecin-resistant phenotype of the parental DT40 cells, further showing that aprataxin directly regulates the cellular sensitivity to camptothecin (Fig. 2).

Low aprataxin tumor levels are associated with good response to irinotecan. We next wanted to investigate the value of aprataxin tumor levels as a marker of response to irinotecan. For this purpose, we used a series of colorectal cancer patients with metastatic disease who received irinotecan-based treatment (see Table 1). A tissue microarray containing duplicate tumor samples from 135 patients was constructed. The levels of expression of aprataxin in these tumors were assessed by immunohistochemistry, and a gradient of expression was observed, ranging from complete absence (Fig. 3A) to high tumor levels of aprataxin (Fig. 3D). The specificity of the antibody was confirmed by Western blotting. A single band of the expected molecular weight was detected in HEK293T cells transiently transfected with an APTX-GFP fusion protein (Supplementary Fig. S2).

In good agreement with previous reports (4, 8), the overall response rate for this series of 135 irinotecan-treated patients with advanced colorectal cancer was 33.6% with a median progression-free and overall survival of 6.7 and 26.3 months, respectively. Fifty-one percent of the cases studied showed low/absent aprataxin, whereas the remaining 49% were categorized as having moderate/high levels (Fig. 3). Aprataxin tumor levels could distinguish two groups of patients with significantly different responses to irinotecan-based treatment. A significantly higher proportion of patients with high aprataxin levels had progressive disease compared with patients with low aprataxin [29 of 57 (50.9%) versus 19 of 59 (32.2%); χ^2 test $P = 0.04$)]. The median progression-free survival of patients with high aprataxin tumor levels was 5.5 months whereas in the group of patients with low aprataxin levels it was 9.2 months (Logrank test $P = 0.03$; hazard ratio, 1.5; 95% confidence interval, 1.04-2.29; Fig. 3E). Moreover, the median overall survival of patients with absent/low aprataxin was 17.7 months longer than in the group of patients with moderate/high aprataxin (19.0 versus 36.7 months, respectively; Logrank test $P = 0.008$; hazard ratio, 2.1; 95% confidence interval, 1.21-3.63; Fig. 3F). In a multivariate analysis with sex, age, tumor grade, and aprataxin levels as covariates, low aprataxin remained a significant marker of better

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/16/8/2375/1995421/2375.pdf by guest on 21 July 2024

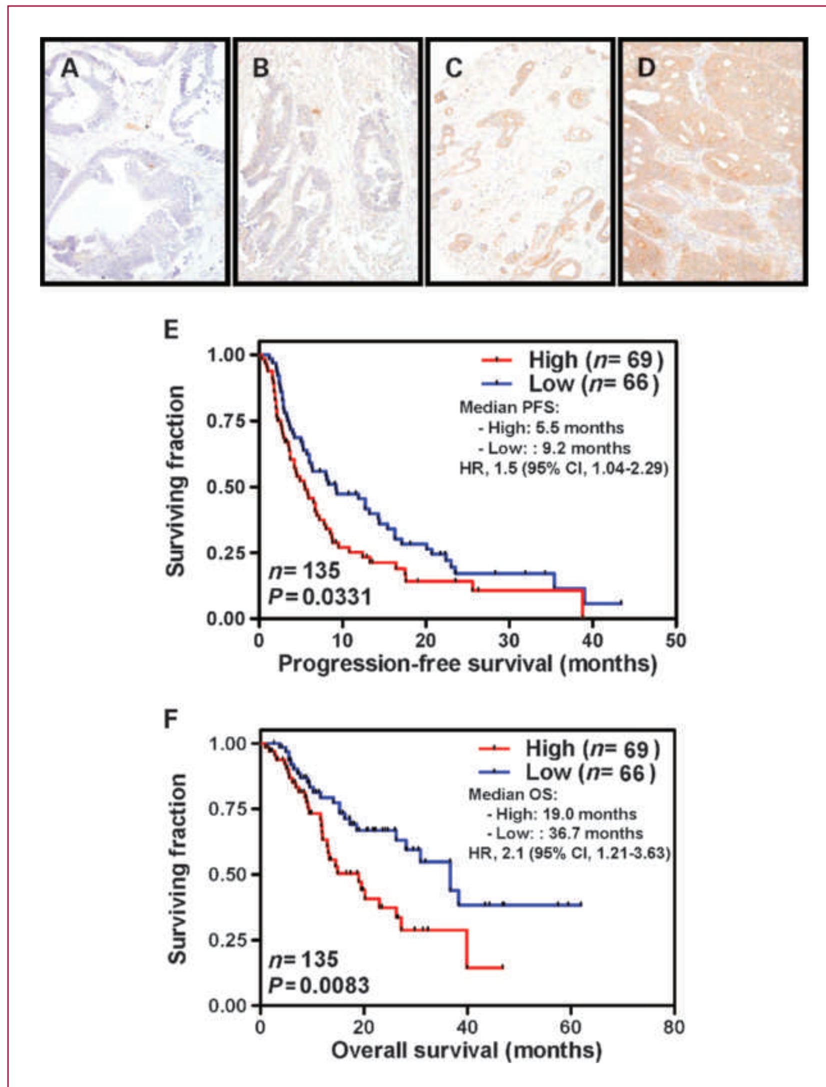

Fig. 3. Aprataxin tumor levels and survival of patients with advanced colorectal cancer receiving irinotecan-based treatment. Immunohistochemical staining of colorectal tumors showed a gradient of expression, with some tumors having no detectable aprataxin (A), high levels (D), or intermediate levels of expression (B and C). Progression-free (E) and overall survival (F) according to aprataxin protein levels are shown (Kaplan-Meier plots). Log-rank *P* values are shown. PFS, progression-free survival; OS, overall survival; HR, hazard ratio; 95% CI, 95% confidence interval.

overall survival in colorectal cancer patients treated with irinotecan (Cox regression; $P = 0.04$), showing a strong association between low aprataxin levels and good response to irinotecan treatment.

***Coding polymorphisms in* APTX.** The coding sequence of *APTX* has six reported polymorphisms (National Center for Biotechnology Information database, September 2009). Five of these are single nucleotide polymorphisms, and in addition, a deletion of 805 bp has been described (see Supplementary Table S2). To investigate the possible association between the sensitivity to camptothecin and the genotype of these *APTX* polymorphisms, we used direct sequencing of 10 different colon cancer cell lines that widely vary in their sensitivity to camptothecin (Supplementary Table S2). We found no variability in the genotype of these genomic regions in the tumor cell lines investigated. Therefore, variations in the coding sequence of *APTX* are not responsible for the expression and sensitivity differences observed in these cell lines, and are unlikely to underlie the differences in tumor response observed in colorectal cancer patients.

Discussion

The availability of molecular markers capable of predicting response to different chemotherapeutic agents used for the treatment of advanced colorectal cancer would constitute a significant step towards a more personalized treatment for these patients. For example, the realization that only patients with wild-type *KRAS* tumor cells are likely to respond to EGFR targeting agents such as cetuximab or panitumumab has significantly improved the management of these patients (9, 30). Patients with mutant *KRAS* tumors are spared from the treatment with EGFR targeting agents and can be treated with alternative agents that are more likely to elicit a positive response. Irinotecan is a TOP1 inhibitor that is frequently used for the treatment of advanced colorectal cancer patients. TOP1 is involved in

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/16/8/2375/1995421/2375.pdf by guest on 21 July 2024

the relaxation of supercoiled DNA occurring during DNA replication or transcription, forming a covalent intermediate with the 3′-terminus of the nicked DNA that is normally released and religated to the adjacent 5′-end. However, treatment with TOP1 inhibitors, such as irinotecan, results in the stabilization of the TOP1-DNA complex that upon collision with the replication fork causes double-strand DNA breaks, cell cycle arrest and death (31). Treatment of advanced colorectal cancer patients with irinotecan as a single agent has shown response rates of approximately 30%, and these rates reach 50% when used in combination with other agents, resulting in significant improvements of progression-free and overall survival of irinotecan-treated patients (4, 8). However, the majority of patients do not show an objective response to irinotecan, and there is therefore an acute need for the identification of molecular markers capable of predicting the probability of response to this agent. Microarray analysis can simultaneously assess the levels of expression of thousands of genes and is therefore a useful tool to screen for potential new molecular markers of response to treatment with chemotherapeutic agents. The use of cell lines allows the rigorous assessment of sensitivity to chemotherapeutic agents individually in a controlled manner, and microarray analysis can then be used to identify genes with expression profiles across multiple tumor cell lines that closely correlate with drug sensitivity. Moreover, the expression profiles identified *in vitro* have been shown to be useful to predict patient response to these agents (16–18).

Here, we used microarray expression analysis to identify apratxin (APTX) as a new molecular marker of response to irinotecan treatment. We found that the levels of apratxin are significantly associated with camptothecin sensitivity in a panel of 30 colorectal cancer cell lines ($P = 0.004$). Apratxin is a member of the histidine triad domain superfamily of nucleotide hydrolases and transferases, and participates in the repair of single- and double-stranded DNA breaks (32). Using an *in vitro* cell system in which both copies of *APTX* have been inactivated by targeted recombination, we showed that low apratxin levels significantly sensitize to camptothecin treatment *in vitro*.

Currently, the best-characterized marker capable of predicting response to irinotecan in patients with advanced colorectal cancer is the direct molecular target TOP1 (14). Irinotecan-treated patients with high TOP1 tumor levels have an overall survival of approximately 14.7 months compared with 17.4 months in patients with high TOP1 levels (14). In this study, we investigated the value of apratxin as a new marker of response to irinotecan treatment. We found that patients with absent/low apratxin tumor levels have better response rates (40.7% versus 26.3%) and longer progression-free (9.2 versus 5.5 months) and overall survival (36.7 versus 19.0 months) than patients with moderate/high apratxin levels. Therefore, tumor levels of apratxin can distinguish two subgroups of patients with advanced colorectal cancer that widely differ in their response to irinotecan treatment. If this result is independently confirmed, ideally on randomized trials, apratxin could be used alone or in combination with other biomarkers to make informed decisions regarding the type of treatment that is likely to be most effective for individual patients. The identification of apratxin as a new marker predicting the probability of response to irinotecan treatment constitutes a new step towards the goal a more individualized treatment for patients with advanced colorectal cancer. For example, a patient with advanced colon cancer with a tumor that has low levels of apratxin, high topoisomerase I, and wild-type *KRAS* would be an ideal candidate to receive combined treatment with irinotecan and cetuximab/panitumumab.

Irinotecan treatment results in the accumulation of DNA strand breaks in tumor cells, and apratxin has been shown to have an important role in the repair of DNA single- and double-strand breaks (12, 21, 22). Although the detailed mechanisms remain to be elucidated, tumors with elevated levels of apratxin would be more resistant to irinotecan because the DNA damage caused by this agent would be repaired more efficiently, and therefore, these patients would have a poor response to this form of treatment. Although several polymorphisms have been reported in the coding sequence of *APTX*, no variants were observed in a series of colon cancer cells with significant differences in their sensitivity to camptothecin. Therefore, coding *APTX* variants are unlikely to play a major role in the response of colorectal tumors to irinotecan.

In conclusion, in this study we show that the histidine triad family protein apratxin directly regulates the cellular sensitivity to camptothecin and the value of assessing apratxin tumor levels as a biomarker capable of predicting response to irinotecan-based treatment in patients with advanced colorectal cancer. Apratxin levels, combined with other markers such as TOP1, could allow identification of a large subset of patients with low probability of response to irinotecan. These patients would be ideal candidates to receive treatment with alternative agents available in an attempt to improve their survival and quality of life.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

We thank Dr. Keith W. Caldecott (Genome Damage and Stability Centre, University of Sussex, Falmer, Brighton) and Dr. Martin F. Lavin (Queensland Institute of Medical Research, Radiation Biology and Oncology, Brisbane, Australia) for kindly providing us with the DT40 APTX KO cell system and the pEGFP-APTX vector, respectively.

Grant Support

Spanish Ministry of Science and Innovation (FIS051394, CP05/00256, SAF2008-00789 and TRA2009-0093) and the Fundación Médica Mutua Madrileña to D. Arango.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received 12/14/2009; revised 01/29/2010; accepted 02/15/2010; published OnlineFirst 04/06/2010.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/16/8/2375/1995421/2375.pdf by guest on 21 July 2024

References

1. Parkin DM, Bray F, Ferlay J, Pisani P. Global cancer statistics, 2002. CA Cancer J Clin 2005;55:74–108.
2. Moertel CG, Fleming TR, Macdonald JS, et al. Fluorouracil plus levamisole as effective adjuvant therapy after resection of stage III colon carcinoma: a final report. Ann Intern Med 1995;122:321–6.
3. Petrelli N, Douglass HO, Jr., Herrera L, et al, Gastrointestinal Tumor Study Group. The modulation of fluorouracil with leucovorin in metastatic colorectal carcinoma: a prospective randomized phase III trial. J Clin Oncol 1989;7:1419–26.
4. Douillard JY, Cunningham D, Roth AD, et al. Irinotecan combined with fluorouracil compared with fluorouracil alone as first-line treatment for metastatic colorectal cancer: a multicentre randomised trial. Lancet 2000;355:1041–7.
5. Goldberg RM, Sargent DJ, Morton RF, et al. A randomized controlled trial of fluorouracil plus leucovorin, irinotecan, and oxaliplatin combinations in patients with previously untreated metastatic colorectal cancer. J Clin Oncol 2004;22:23–30.
6. Cunningham D, Humblet Y, Siena S, et al. Cetuximab monotherapy and cetuximab plus irinotecan in irinotecan-refractory metastatic colorectal cancer. N Engl J Med 2004;351:337–45.
7. Hurwitz H, Fehrenbacher L, Novotny W, et al. Bevacizumab plus irinotecan, fluorouracil, and leucovorin for metastatic colorectal cancer. N Engl J Med 2004;350:2335–42.
8. Saltz LB, Cox JV, Blanke C, et al, Irinotecan Study Group. Irinotecan plus fluorouracil and leucovorin for metastatic colorectal cancer. N Engl J Med 2000;343:905–14.
9. Allegra CJ, Jessup JM, Somerfield MR, et al. American Society of Clinical Oncology provisional clinical opinion: testing for KRAS gene mutations in patients with metastatic colorectal carcinoma to predict response to anti-epidermal growth factor receptor monoclonal antibody therapy. J Clin Oncol 2009;27:2091–6.
10. Aschele C, Lonardi S, Monfardini S. Thymidylate synthase expression as a predictor of clinical response to fluoropyrimidine-based chemotherapy in advanced colorectal cancer. Cancer Treat Rev 2002;28:27–47.
11. Shirota Y, Stoehlmacher J, Brabender J, et al. ERCC1 and thymidylate synthase mRNA levels predict survival for colorectal cancer patients receiving combination oxaliplatin and fluorouracil chemotherapy. J Clin Oncol 2001;19:4298–304.
12. Hsiang YH, Hertzberg R, Hecht S, Liu LF. Camptothecin induces protein-linked DNA breaks via mammalian DNA topoisomerase I. J Biol Chem 1985;260:14873–8.
13. Kohne CH, van Cutsem E, Wils J, et al. Phase III study of weekly high-dose infusional fluorouracil plus folinic acid with or without irinotecan in patients with metastatic colorectal cancer: European Organisation for Research and Treatment of Cancer Gastrointestinal Group Study 40986. J Clin Oncol 2005;23:4856–65.
14. Braun MS, Richman SD, Quirke P, et al. Predictive biomarkers of chemotherapy efficacy in colorectal cancer: results from the UK MRC FOCUS trial. J Clin Oncol 2008;26:2690–8.
15. Koopman M, Knijn N, Richman S, et al. The correlation between topoisomerase-I (Topo1) expression and outcome of treatment with capecitabine and irinotecan in advanced colorectal cancer (ACC) patients (pts) treated in the CAIRO study of the Dutch Colorectal Cancer Group (DCCG). Eur J Cancer Suppl 2009;7:321.
16. Lee JK, Havaleshko DM, Cho H, et al. A strategy for predicting the chemosensitivity of human cancers and its application to drug discovery. Proc Natl Acad Sci U S A 2007;104:13086–91.
17. Arango D, Laiho P, Kokko A, et al. Gene-expression profiling predicts recurrence in Dukes' C colorectal cancer. Gastroenterology 2005;129:874–84.
18. Mariadason JM, Arango D, Shi Q, et al. Gene expression profiling-based prediction of response of colon carcinoma cells to 5-fluorouracil and camptothecin. Cancer Res 2003;63:8791–812.
19. Moreira MC, Barbot C, Tachi N, et al. The gene mutated in ataxia-ocular apraxia 1 encodes the new HIT/Zn-finger protein aprataxin. Nat Genet 2001;29:189–93.
20. Date H, Onodera O, Tanaka H, et al. Early-onset ataxia with ocular motor apraxia and hypoalbuminemia is caused by mutations in a new HIT superfamily gene. Nat Genet 2001;29:184–8.
21. Gueven N, Becherel OJ, Kijas AW, et al. Aprataxin, a novel protein that protects against genotoxic stress. Hum Mol Genet 2004;13:1081–93.
22. Mosesso P, Piane M, Palitti F, Pepe G, Penna S, Chessa L. The novel human gene aprataxin is directly involved in DNA single-strand-break repair. Cell Mol Life Sci 2005;62:485–91.
23. Skehan P, Storeng R, Scudiero D, et al. New colorimetric cytotoxicity assay for anticancer-drug screening. J Natl Cancer Inst 1990;82:1107–12.
24. Arango D, Corner GA, Wadler S, Catalano PJ, Augenlicht LH. c-myc/p53 interaction determines sensitivity of human colon carcinoma cells to 5-fluorouracil *in vitro* and *in vivo*. Cancer Res 2001;61:4910–5.
25. Arango D, Mariadason JM, Wilson AJ, et al. c-Myc overexpression sensitises colon cancer cells to camptothecin-induced apoptosis. Br J Cancer 2003;89:1757–65.
26. Ahel I, Rass U, El-Khamisy SF, et al. The neurodegenerative disease protein aprataxin resolves abortive DNA ligation intermediates. Nature 2006;443:713–6.
27. Therasse P, Arbuck SG, Eisenhauer EA, et al, European Organization for Research and Treatment of CancerNational Cancer Institute of the United StatesNational Cancer Institute of Canada. New guidelines to evaluate the response to treatment in solid tumors. J Natl Cancer Inst 2000;92:205–16.
28. Graham FL, van der Eb AJ. A new technique for the assay of infectivity of human adenovirus 5 DNA. Virology 1973;52:456–67.
29. Davalos V, Dopeso H, Castano J, et al. EPHB4 and survival of colorectal cancer patients. Cancer Res 2006;66:8943–8.
30. Amado RG, Wolf M, Peeters M, et al. Wild-type KRAS is required for panitumumab efficacy in patients with metastatic colorectal cancer. J Clin Oncol 2008;26:1626–34.
31. Xu Y, Villalona-Calero MA. Irinotecan: mechanisms of tumor resistance and novel strategies for modulating its activity. Ann Oncol 2002;13:1841–51.
32. Caldecott KW. Single-strand break repair and genetic disease. Nat Rev Genet 2008;9:619–31.

Downloaded from http://aacrjournals.org/clincancerres/article-pdf/16/8/2375/1995421/2375.pdf by guest on 21 July 2024