

Different proteome pattern of epidermal growth factor receptor–positive colorectal cancer cell lines that are responsive and nonresponsive to C225 antibody treatment

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Abstract

The monoclonal antibody C225 directed against the epidermal growth factor receptor (EGFR) blocks downstream mitogenic signaling and is effective in patients with advanced colorectal cancer. Clinical data, however, suggest the presence of primary and secondary resistance mechanisms that are hardly understood. To define proteins involved in EGFR-triggered growth regulation and potential resistance mechanisms, we characterized the proteome profile of two colorectal cancer cell lines with a high expression of functional EGFR but a different response to treatment with C225. In Caco-2 and HRT-18, a complete saturation of EGFR was achieved after incubation with C225; whereas Caco-2 showed inhibition of proliferation, growth of HRT-18 was not suppressed. Using two-dimensional electrophoresis and subsequent mass spectrometry, we identified 14 proteins differentially expressed in both cell lines. All proteins are involved in metabolic pathways and malignant growth. Expression of enzymes such as ubiquitin carboxyl-terminal hydrolase isozyme 1, glutathione S-transferase P, and chloride intracellular channel protein 1 does not seem to interfere with the antiproliferative effect of anti-EGFR antibody. On the other hand, expression of proteins such as fatty acid binding protein and heat shock protein 27 might constitute strong antiapoptotic effects contributing to the

nonresponse of HRT-18 to C225 treatment. Proteome-based investigations can help us better understand the complex protein interactions involved in EGFR signaling and its blockage by therapeutic monoclonal antibodies. [Mol Cancer Ther 2004;(3)12:1551–8]

Introduction

Colorectal cancer represents one of the most common malignancies worldwide (1), with ~20% of patients already presenting with distant metastases at diagnosis (2). Although disease control can be achieved by cytotoxic drugs in a considerable number of patients, no curative chemotherapy regimen is available due to primary or secondary resistance mechanisms. Therefore, there is a need for novel therapeutic strategies that may improve the outcome of these patients. Expression of growth factors and their receptors are key elements in the pathogenesis and maintenance of malignant growth (3). In colorectal cancer, epidermal growth factor (EGF) receptor (EGFR) is expressed in ~70% of patients (4), suggesting an essential role in autocrine growth mechanisms. EGFR is a transmembrane glycoprotein consisting of an extracellular ligand binding domain, a transmembrane region, and an intracellular protein tyrosine kinase domain (5). The natural ligands are EGF and transforming growth factor- α , which bind to the extracellular domain of EGFR and activate the receptor and its downstream signal transduction pathways, ultimately causing activation or modulation of cellular processes such as differentiation, angiogenesis, growth, and survival (6). EGFR expression correlates with tumor progression, resistance to radiotherapy and chemotherapy, and poor prognosis (7, 8).

Several agents such as monoclonal antibodies (mAb; ref. 9) or low molecular weight tyrosine kinase inhibitors (10) have been designed to specifically block EGFR signaling. mAbs against the extracellular domain of EGFR compete with its natural ligand for receptor binding, thereby preventing kinase activation (11). The human-mouse chimeric mAb C225 has a high affinity for EGFR and is currently in phase II and III clinical trials in colorectal cancer, head and neck cancer, and several other tumor types (9). Clinical efficacy of C225 seems to involve multiple mechanisms, such as inhibition of cell cycle progression, induction of apoptosis, inhibition of angiogenesis, and inhibition of metastasis (12). Furthermore, binding of C225 to its target may induce immune effector mechanisms such as antibody-dependent cell-mediated cytotoxicity and complement-dependent cytotoxicity (13). Clinical trials completed in EGFR-positive metastatic

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colorectal cancer have thus far shown activity of C225 in combination with chemotherapy in patients who failed to respond to conventional anticancer therapy (14, 15). When given as monotherapy, C225 induces response in a limited number of patients only (14), suggesting the presence of resistance mechanisms that interfere with EGFR signal transduction pathways. The existence of these mechanisms is supported by *in vitro* data that show that EGFR expression does not necessarily correlate with sensitivity to EGFR antagonists (16). Although resistance of cancer cells to chemotherapy is caused by multiple factors, such as *MDR1* gene expression (17) or Bcl-2 and Akt overexpression (16, 18), potential mechanisms of mAb resistance are hardly understood.

To identify proteins involved in resistance mechanisms against mAb therapy, we selected two colorectal cancer cell lines with a high expression of EGFR but a different sensitivity toward treatment with C225. By comparing the proteomic profile of the Caco-2 and HRT-18 cell lines after two-dimensional PAGE, significant differences in the expression of 14 proteins were detected. When these proteins were identified using liquid chromatography-nanospray mass spectrometry, it was seen that most of them are involved in cell function and metabolism. Our data suggest that proteome-based technologies represent a new tool for understanding the complex protein network and its interactions in malignant cells after treatment with anticancer agents such as mAbs.

Materials and Methods

Cell Culture and Treatment with C225 Antibody

The Caco-2 and HRT-18 colorectal cancer cell lines were purchased from the American Type Culture Collection (Manassas, VA). Caco-2 cells were grown in Eagle's MEM (PAA Laboratories GmbH, Linz, Austria) supplemented with 2 mmol/L L-glutamine, 1.5 g/L sodium bicarbonate, 0.1 mmol/L nonessential amino acids, 50 units/mL penicillin, 50 µg/mL streptomycin, and 10% FCS (Sigma-Aldrich, Vienna, Austria). HRT-18 cells were maintained in RPMI 1640 (PAA Laboratories) containing 2 mmol/L L-glutamine, 50 units/mL penicillin, 50 µg/mL streptomycin, and 10% FCS. Cultures were incubated in a 5% CO₂ humidified atmosphere.

C225, a human-mouse chimeric anti-EGFR IgG1 class mAb (provided by Merck KGaA, Darmstadt, Germany) was used at 10 µg concentration.

For statistical evaluation, mean values and SD were calculated using three independent experiments; significance was determined by paired Student *t* test.

Analysis of EGFR Expression

For analysis of EGFR expression by flow cytometry (FACSCalibur, Becton Dickinson, San Jose, CA), 1×10^6 cells per tube were pelleted at $200 \times g$, washed twice with PBS, and stained with 4 µg/mL mouse monoclonal IgG2a anti-EGFR (528) phycoerythrin: sc-107 phycoerythrin antibody (Santa Cruz Biotechnology, Heidelberg, Germany). An equivalent amount of phycoerythrin-conju-

gated IgG2a mAb (DakoCytomation GmbH, Vienna, Austria) was used as an isotype control. Propidium iodide (Sigma Chemical Co., St. Louis, MO) was added to all assays to exclude dead cells. From each sample, 10,000 life events were collected by FACSCalibur.

Blocking of EGFRs with C225 Antibody

After detachment from culture plates using trypsin, 5×10^5 cells were aliquoted and mixed with 10 µg/mL C225 mAb or IgG1 (10 µg/mL, DakoCytomation) as a negative control. After an incubation period of 1, 5, 15, or 25 minutes, cells were washed twice with 1 mL PBS (pH 7.5) and centrifuged at $200 \times g$ for 7 minutes. Then, cells were incubated with the anti-EGFR (528) phycoerythrin: sc-107 phycoerythrin antibody for 15 minutes at room temperature, and kinetic binding variables as well as blocking of EGFR with C225 were determined by fluorescence-activated cell sorting analysis.

Proliferation Assay

Cultured cells with and without C225 treatment were fixed after 0, 24, 48, or 72 hours with 50 µL/well ice-cold 50% trichloroacetic acid at 4°C overnight. After washing five times with water and air-drying for ~20 minutes, cells were stained with 100 µL of 0.4% sulforhodamine B (Sigma-Aldrich, Vienna, Austria) in 1% acetic acid for 15 minutes. Subsequently, plates were washed five times with 1% acetic acid and air-dried and the dye was resuspended in 100 µL of 10 mmol/L Tris buffer (pH 10.5). Dye quantification was done by a microplate reader (SpectraFLUOR Plus, Tecan, Austria) at 510 nm. Cell proliferation was determined as percentage of control.

Western Blot Analysis

For evaluation of the EGFR phosphorylation status by Western blotting, cells were lysed for 15 minutes at 4°C in radioimmunoprecipitation assay [50 mmol/L Tris-HCl (pH 7.5), 150 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 1 µg/mL leupeptin, 5 µg/mL aprotinin, 1% NP40, 0.5% deoxycholic acid sodium salt, 0.1% SDS] and sonicated. After centrifugation at $12,000 \times g$ for 10 minutes at 4°C, supernatant was collected and protein concentrations were determined by a commercial protein assay (Bio-Rad Laboratories, Hercules, CA); 40 µg of protein per lane were separated by 12% SDS-PAGE and electroblotted onto nitrocellulose membranes (Schleicher & Schuell, Dassel, Germany). Protein loading was controlled by Ponceau S red staining of membranes. After blocking for 1 hour in TBS supplemented with 5% nonfat milk and 0.1% Tween 20 (Sigma-Aldrich Vienna, Austria), membranes were incubated for 1 hour at room temperature with antibody against the activated form of human EGFR (BD Biosciences, San Diego, CA) or with α -tubulin (Oncogene Research, Cambridge, MA) as loading control. Membranes were washed thrice in TBS-Tween 20 and incubated for 1 hour with horseradish peroxidase-conjugated anti-mouse secondary antibody (Amersham, Les Ulis, France). Immunodetection was done with an enhanced chemiluminescence system (Amersham Biosciences, Vienna, Austria).

Two-dimensional PAGE

For sample preparation, Caco-2 and HRT-18 cells were scraped and harvested by centrifuging at 4°C for 10 minutes at 200 × g. After washing twice with PBS, cells were lysed for 15 minutes at 4°C in lysis buffer [10 mmol/L Tris-HCl (pH 7.5), 25 mmol/L NaCl, 1 mmol/L EDTA, 1 mmol/L phenylmethylsulfonyl fluoride, 1 µg/mL leupeptin, 5 µg/mL aprotinin, 0.1% NP40] and sonicated. To remove the nuclear fraction, the homogenate was centrifuged at 3,000 × g for 10 minutes at 4°C and the supernatant collected in a new tube. To avoid contamination with salts or nucleic acids, lysates were processed using trichloroacetic acid at a final concentration of 10% (Serva, Heidelberg, Germany). Precipitated proteins were washed thrice with cold acetone and resuspended with isoelectric lysis solution (7 mol/L urea, 2 mol/L thiourea, 4% CHAPS, 0.5% immobilized pH gradient buffer). Protein concentrations were determined with a commercial protein assay (Bio-Rad Laboratories).

For first-dimension isoelectric focusing, 60 µg of protein per sample were diluted to 450 µL with rehydration buffer and loaded on immobilized pH gradient strips. Active rehydration (50 V) was carried out at 20°C for 12 hours. For pH 3 to 10 immobilized pH gradient strips, isoelectric focusing was done at 250 V for 30 minutes, 500 V for 1 hour, 2,000 V for 1 hour, and 8,000 V for 1 hour until 64,000 V were reached in total. pH 4 to 7 and 6 to 9 immobilized pH gradient strips were processed at 250 V for 30 minutes, 500 V for 1 hour, 2,000 V for 1 hour, and 8,000 V for 1 hour until 110,000 V.

For second-dimension isoelectric focusing, samples were separated on 12.5% polyacrylamide gels with the Ettan

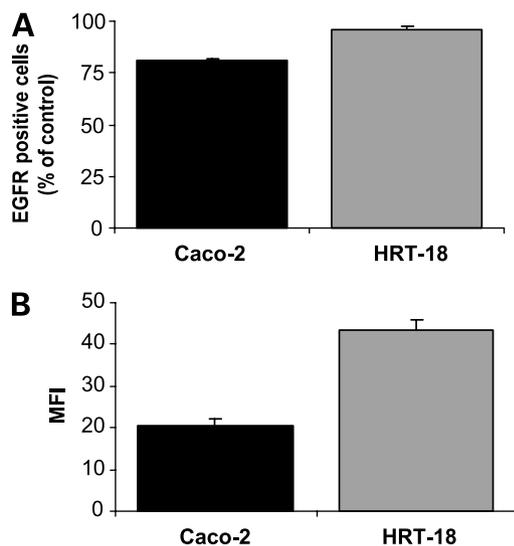


Figure 1. EGFR expression in Caco-2 and HRT-18 cells. Cells were stained for the presence of EGFR and evaluated by fluorescence-activated cell sorting analysis. **A**, percentage of the EGFR-positive cell population; **B**, mean fluorescence intensity of EGFR staining. *Columns*, mean of three independent experiments; *bars*, SD.

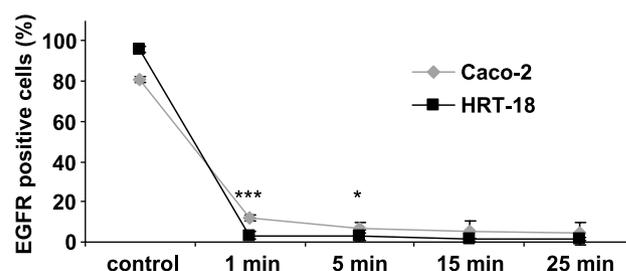


Figure 2. Binding of C225 to EGFR in Caco-2 and HRT-18 cells. Cells were incubated with C225 (10 µg) for 0 to 25 minutes and free EGFR epitopes were analyzed by flow cytometry. No free epitopes were detected in either cell line after 20 minutes. *Points*, mean of three independent experiments; *bars*, SD. *, $P < 0.05$; ***, $P < 0.001$.

DALTwelve System following the standard procedure recommended by the manufacturer (Amersham Biosciences, Vienna, Austria). After electrophoresis, gels were silver-stained and scanned using an ImageScanner (Amersham Pharmacia Biotech AB, Uppsala, Sweden). Data analysis was done with ImageMaster 2D Elite version 4.01 software (Amersham Pharmacia Biotech, Uppsala, Sweden). To obtain a standard gel for each individual cell line, background subtraction, spot detection, and matching were done using gels from three runs. These standard gels were then matched to yield information about differentially expressed proteins. Normalized spot volume was used in all our studies.

Identification of Proteins by Mass Spectrometry

Protein analysis was done as published previously (19). Protein digests were separated using capillary high-performance liquid chromatography connected online to a LCQ ion trap instrument (ThermoFinnigan, San Jose, CA) equipped with a nanospray interface. Tandem mass spectrometry spectra were searched against a human database using SEQUEST (LCQ BioWorks, ThermoFinnigan).

Results

EGFR Expression and Blocking of EGFR with C225 Antibody

Both cancer cell lines revealed high expression of EGFR (Fig. 1A), with 80.6 ± 1.6% of Caco-2 cells and 95.7 ± 1.7% of HRT-18 cells being positive. Mean fluorescence intensity for EGFR staining was 20.35 ± 1.8 for untreated Caco-2 cells and 43.6 ± 2.3 for HRT-18 cells (Fig. 1B). To investigate the ability of EGFRs on both cell lines to bind to anti-EGFR mAb, cells were treated with C225. The level of EGFRs free of C225 was measured by flow cytometry using a phycoerythrin-conjugated antibody. Kinetics of C225 binding are provided in Fig. 2. During the first minute of the incubation period, the amount of free epitopes of EGFR decreased rapidly. The Caco-2 cell line exhibited slightly slower binding of C225 as compared with HRT-18 cells. After 1 minute of treatment with C225, 12 ± 1.4% Caco-2 cells and only 3.16 ± 1.9% HRT-18 cells expressed free epitopes. In neither cell line were free epitopes observed

after 25 minutes of incubation with the conjugated anti-EGFR mAb. These results indicate a high binding rate of anti-EGFR mAb in Caco-2 and HRT-18 colorectal cancer cell lines.

Functional EGFR Activation

To determine the functional activity of EGFRs, cell lines were stimulated with EGF (10 nmol/L) and phosphorylation status of EGFR was evaluated by Western blotting. Results of immunoblotting with an antibody specifically directed against the phosphorylated EGFR on Caco-2 and HRT-18 cells are shown in Fig. 3. Maximum phosphorylation of EGFR in Caco-2 cells was achieved after 10 minutes and in HRT-18 cells after 20 minutes.

Growth Inhibitory Effect of C225

When Caco-2 and HRT-18 cells were incubated with C225 for 24, 48, or 72 hours, each cell line exhibited a completely different growth pattern. Whereas treatment with C225 at a concentration of 10 μ g resulted in time-dependent growth inhibition of Caco-2 cells with $91.2 \pm 4.14\%$ of cells surviving after 24 hours, $71.03 \pm 4.97\%$ after 48 hours, and $56.6 \pm 3.07\%$ after 72 hours, growth of HRT-18 cells was not inhibited at all as compared with untreated control cells (Fig. 4). In addition, a further increase in C225 concentration to up to 100 μ g did not result in growth inhibition of HRT-18 cells (data not shown).

Identification of Proteins Using Two-dimensional PAGE

To identify proteins with different levels of expression in Caco-2 and HRT-18 cells, we examined the protein profiles of whole cell extracts after removal of the nuclear fraction by two-dimensional PAGE. Silver-stained gels were analyzed with ImageMaster 2D Elite version 4.01 software for intensity of protein spots. Mean and SD values of relative spot volumes and differences in expression were calculated using at least three independent experiments. When the expression level of individual proteins in the two cell lines differed by a ratio of >2.0 , spots were quantified according to their relative volumes. Among the $\sim 1,900$ protein spots

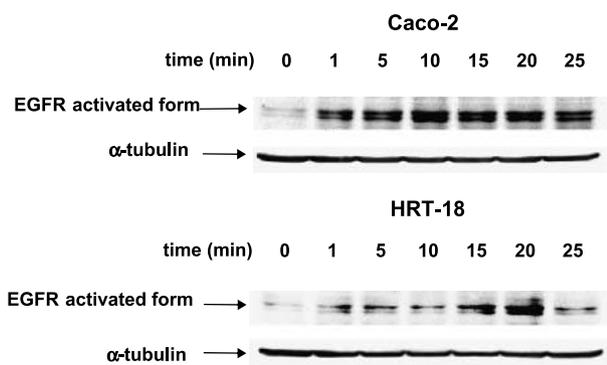


Figure 3. Time-dependent activation of EGFR on Caco-2 and HRT-18 cells. Cells were serum starved overnight followed by 10 nmol/L EGF stimulation. Samples for Western blot analysis were collected before treatment and at 1, 5, 10, 15, 20, and 25 minutes after EGF stimulation. Maximum phosphorylation of EGFR was achieved in Caco-2 cells after 10 minutes and in HRT-18 cells after 20 minutes.

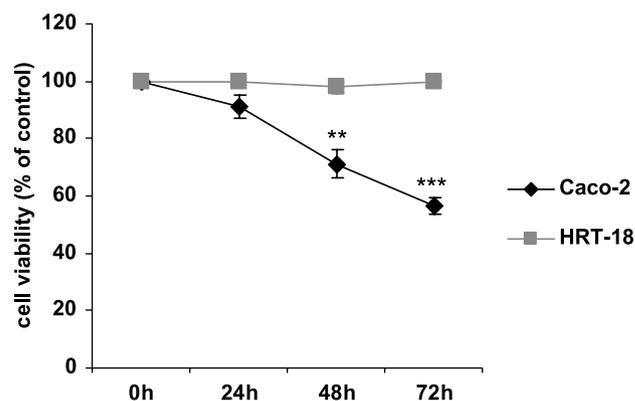


Figure 4. Effect of C225 on the cell growth of EGFR-positive Caco-2 and HRT-18 cells. Cells were treated with 10 μ g C225 for 0 to 72 hours. Only growth of Caco-2 cells was inhibited significantly after C225 treatment. Points, mean of three independent experiments; bars, SD. **, $P < 0.01$; ***, $P < 0.001$ versus cells incubated without C225.

detected per cell line, 14 proteins revealed this difference and also had a sufficient level of expression to allow further processing. Twelve of these proteins were detected in both cell lines with a varying degree of expression, whereas two others were present in one cell line only. All these proteins were analyzed using mass spectrometry in conjunction with the Swiss-2DPAGE and Siena-2-DPAGE protein databases to assign putative identities.

Identified proteins as well as their physical variables and biological functions are summarized in Tables 1 and 2. They belong to different structural or functional families such as proteins with detoxification function, metabolic enzymes, cytoskeleton-related proteins, cell cycle regulators, chaperones, and proteins with calcium channel activity as well as with unknown functions. The expression level of none of these proteins was changed after treatment with C225.

Discussion

The increasing understanding of the role of growth factors and their receptors in the generation and progression of malignant disease has led to the development of numerous drugs targeting specific molecular structures on cancer cells. Clinical trials of anti-EGFR mAb C225 in EGFR-positive colorectal carcinoma patients have shown that the antibody can be active when given alone or in combination with chemotherapy (20–23). However, remissions are usually of limited duration and occur only in some 10% of patients treated with the antibody alone (14), suggesting the existence of primary and secondary resistance mechanisms.

To define proteins that might be involved in the occurrence of resistance against therapeutic mAbs, we investigated two colorectal cancer cell lines with a high expression of functional EGFR. In Caco-2 and HRT-18 cells, a complete saturation of EGFR was achieved after incubation with C225; whereas growth of Caco-2 cells was inhibited in a time-dependent fashion, proliferation of HRT-18 cells was not significantly influenced. Both cell

Table 1. Characteristics of proteins identified by two-dimensional PAGE

Target protein	Accession no.	Sequence coverage (%)	MW (kDa)	Caco-2	HRT-18
Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1)	P09936	70.5	24.8		
Chloride intracellular channel protein 1 (CLIC 1)	O00299	52.9	26.9		
Glutathione-S-transferase P (GSTP)	P09211	66.3	23.4		
Nicotinate-nucleotide pyrophosphorylase (QPRTase)	Q15274	35.6	30.8		
Microtubule-associated protein RP/EB family member 1	Q15691	59.3	30.0		
Annexin A3	P12429	59.4	36.4		
Galectin-3	P17931	34.2	26.2		
Protein kinase C inhibitor protein-1 (PKCI-1)	P31946	46.6	27.9		
Epidermal fatty acid binding protein (E-FABP)	Q01469	76.4	15.2		
Heat shock protein 27-kDa (HSP 27)	P04792	44.1	22.8		
Profilin I	P07737	32.4	15.1		
Inorganic pyrophosphatase (Ppase)	Q15181	62.4	32.7		
Phosphoserine aminotransferase (PSAT)	Q9Y617	46.8	40.4		
Proteasome subunit α type 7 (PSA 7)	O14818	43.5	27.9		

NOTE: Protein identities, accession number, sequence coverage, molecular weight (kDa), and proteins visualized by silver staining are summarized. Arrows represent differential protein expression in cells. Abbreviation: MW, molecular weight.

lines revealed a high expression of functional EGFR, suggesting a comparable potential to induce growth factor signaling. Phosphorylation of EGFR in HRT-18 cells, however, was delayed as compared with Caco-2 cells, suggesting an influence from other factors that are not yet understood. Therefore, proteins involved in EGFR phosphorylation as well as in signal transduction downstream of EGFR are potentially involved in growth regulation and the generation of resistance against C225 in HRT-18 cells.

Our knowledge of resistance mechanisms against mAb therapy is still limited. Recently, it was shown that activation of mitogen-activated protein kinase kinase/extracellular signal-regulated kinase and phosphatidylinositol 3'-kinase/Akt pathways may protect cells from apoptosis induced by EGFR-targeted agents (24, 25).

Inhibition of EGFR signaling by therapeutic mAbs, however, can occur at various levels, and not all of them are well understood. Several antitumor mechanisms have been defined and are thought to act in concert: inhibition of cell cycle progression (26, 27), induction of apoptosis (28, 29), antiangiogenic and antimetastatic effects (30–32), and immunomodulation (13). As resistance may thus occur at various levels with multiple factors being involved, we aimed to define a spectrum of markers that are differentially expressed in Caco-2 and HRT-18 cells. Analysis of the proteome profile of both cell lines by two-dimensional PAGE revealed 14 proteins with a highly significant difference in expression level. All these proteins are involved in metabolic pathways, and several of them have been shown previously to be involved in malignant growth.

Table 2. Function and expression of identified proteins

Protein	Function	Caco-2 / HRT-18 expression
UCH-L1	Involved in the translational processing of pro-ubiquitin gene products as well as in the release of ubiquitin from tagged proteins	Expressed in Caco-2
CLIC 1	Belongs to the superfamily of GST, is a vital component of cellular detoxification	4.3/1.0
GSTP	Demonstrates various functional activities, such as regulation of stress kinases, cell protection from cytotoxic and radiation damage, and participation in carcinogenesis	47.2/1.0
QPRTase	Regulates tryptophane biosynthesis and probably participates in the ordering of membrane permeability	5.1/1.0
Microtubule-associated protein RP/EB family member 1	Participates in regulating microtubule dynamics and chromosome segregation	2.8/1.0
Annexin A3	Involved in membrane trafficking, fusion, and permeabilization	2.0/1.0
Galectin-3	Implicated in cell growth, differentiation, adhesion, RNA progression, and apoptosis	4.1/1.0
PKCI-1	Although it was originally thought to inhibit PKC its actual physiologic function is not known	3.4/1.0
E-FABP	Modulation of mitosis, cell growth, differentiation, PKC, activation, sequestration, or removal of cytotoxic drugs	Expressed in HRT-18
HSP 27	Inhibits actin polymerization, prevents pro-caspase-9 and pro-caspase-3 activation, and regulates detoxification mechanisms mediated by the action of glutathione	1.0/4.5
Profilin I	G-actin sequestration and inhibition actin polymerization	1.0/3.2
Ppase	Plays an important role in energy metabolism, providing a thermodynamic pull for many biosynthetic reactions. Very little is known about the enzyme from mammalian tissues	1.0/3.3
PSAT	Participates in cell metabolism and cellular replication	1.0/20.9
PSA 7	Participates in positive or negative regulation of transcriptional factors in cancer cells	1.0/5

NOTE: Differences in protein expression are indicated as ratio between means of protein expression in Caco-2 and HRT-18 cells. For key to abbreviations, see Table 1.

Ubiquitin carboxyl-terminal hydrolase isozyme L1 (UCH-L1) was exclusively expressed in Caco-2 cells. The enzyme can serve as a marker for invasive colorectal carcinoma and as a prognostic factor in pancreatic and lung cancer (33–35). Recently, it was shown that UCH-L1 enzymatic activity is antiproliferative, suggesting that its expression may be a response to tumor growth (36). Two other proteins, glutathione *S*-transferase P (GSTP) and chloride intracellular channel protein 1 (CLIC1), showed a significantly higher expression in Caco-2 cells. Both enzymes belong to the glutathione *S*-transferase superfamily and exert various functions, such as regulation of stress kinases (37), protection of cells from apoptosis (38), involvement in the development of ovarian and colorectal carcinoma (39, 40), and protection of cells from cytotoxic and radiation damage (41, 42). Furthermore, GSTP is associated with poor prognosis and survival in glioma

patients (43). The exclusive or significantly higher expression of all these enzymes in Caco-2 cells suggests that they do not interfere with the antiproliferative effect of the anti-EGFR antibody. Whether this is also applicable for the other proteins with a higher expression level in Caco-2 (Tables 1 and 2) is unclear due to our limited knowledge of their potential role in malignant growth.

In principle, all proteins expressed at a higher level in cells nonsensitive to C225 may be involved in resistance mechanisms. Although several of these proteins are expressed in both cell lines, the epidermal fatty acid binding protein is present only in HRT-18 cells. The protein is involved in modulation of mitosis, cell growth, and differentiation (44). Fatty acids are transported by fatty acid binding protein and hydroxylated. Hydroxylated fatty acids modulate the EGF signaling pathways and stimulate both EGFR and protein kinase C (PKC; ref. 45). As PKC is

a mediator of the phosphatidylinositol 3'-kinase/Akt pathway that prevents cells from apoptosis, increased expression of epidermal fatty acid binding protein may be involved in mechanisms contributing to the nonresponse of HRT-18 to C225 treatment. It is interesting to note that the PKC inhibitor protein-1 is expressed less in HRT-18 than in Caco-2 cells.

Up-regulation of PKC activity also leads to phosphorylation of heat shock protein 27 (HSP 27; ref. 46), an antiapoptotic protein expressed more in HRT-18 cells. HSP 27 is a cytoplasmic chaperone participating in stress resistance, cell growth and differentiation, microfilament organization, and assembly of polypeptides (47, 48). Overexpression of HSP 27 not only is associated with aggressive behavior of various tumors and with patient survival but also correlates with resistance to chemotherapeutic agents (49).

In summary, proteome-based technologies represent an important tool for understanding the complex protein network and its interactions in cancer. The proteomic profile of cancer cell lines can contribute to our understanding of growth regulation and its modulation by investigational drugs. The different expression of proteins can guide us to a more detailed investigation of the physiologic and pathologic role of these proteins and their potential involvement in resistance mechanisms.

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