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Filter-Grown Caco-2 Cell Proteome: A Special Emphasis on Drug Disposition Proteins

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Abstract

Research on the physiology of intestinal cells and drug transport often makes use of Caco-2 cells. Here, we compared the proteomes of the human colon and jejunum with those of filter-grown Caco-2 cells by quantifying their global proteome using the total protein technique. We found 8096 proteins in all. Extensive research on proteins that regulate enterocyte differentiation—such as integrin, adherens and tight junctions, and brush-border hydrolases—provided almost exhaustive coverage of the predicted proteins. One hundred twelve solute carriers and twenty ATP-binding cassette transporters were among the 327 proteins found to be involved in absorption, distribution, metabolism, and excretion. The levels of OATP2B1 were sixteen times more in Caco-2 cells compared to jejunum. At clinically relevant intestine concentrations, OATP2B1 accounted for 60%-70% of the uptake kinetics of pitavastatin, an OATP2B1 substrate, in Caco-2 monolayers. We aimed to understand how this discrepancy affected in vitro-in vivo extrapolations. Together, pitavastatin kinetics and transporter concentrations were used to simulate the role of active transport and membrane penetration in the jejunum. Pitavastatin absorption in vivo is mostly mediated via transmembrane diffusion, as shown by the much decreased transporter contribution (<5%) caused by the lower OATP2B1 expression in the jejunum. Finally, we provide the first comprehensive quantification of the filter-grown Caco-2 proteome. To correctly interpret drug transport pathways in the human gut, we also show that transporter expression levels are very important.

Introduction

The colon cancer in humans Pogh et al. were the first to isolate the Caco-2 cell line.1 In their publication on the spontaneous differentiation of filter-grown Caco-2 monolayers to an enterocyte-like phenotype, Zweibaum et al. presented the use of this cell type for investigations of the physiology of epithelial cells.2,3 Because of this, researchers in the pharmaceutical industry were able to use this cell line to examine how solutes are transported and absorbed.4–7 in Using Caco-2 cells, Borchardt and Wilson were the pioneers in studying active transport mechanisms, such as bile transfer.

proteins, vitamins, amino acids, and peptides.6,8-10 Since methods for identifying or knocking off the target transporter were just recently developed, functional studies dominated their groundbreaking work and the subsequent many investigations. Modern mass spectrometry has made it feasible to map the whole Caco-2 cell proteome.11 Gene editing tools like CRISPR-Cas913 may entirely knock out proteins of relevance to better understand their function, and targeted proteomics can quantify collections of proteins, including key drug transporters12. Modeling transport capacity in the intricate cellular environment may be achieved by integrating systems biology methods with protein expression and kinetic characteristics.14protein expression profile with that of the human jejunum and colon, with a particular emphasis on ADME proteins (transporters, drug-metabolizing enzymes, and others) that are important for drug disposal processes. In the end, we modeled the jejunal drug permeability via the high affinity and low capacity transporter OATP2B1 (SLCO2B1), which is thought to play a significant role in the absorption of anionic drug substrates in the intestines, using the protein concentration data.15-17

Methods

Supply items

The chemicals and cell culture medium were all bought from Sigma-Aldrich or Thermo Fisher Scientific, respectively, located in Waltham, MA and St. Louis, MO. Corning (Corning, NY) supplied the cell culture plates and Trans well permeable filters' do research on cell culture.

From passage 95 to 105, Caco-2 cells were cultured in Dulbecco's modified Eagle's medium with 10% fetal calf serum and 1% non-essential amino acids. The cells were initially received from the American Type Culture Collection. Following the methods previously mentioned, they were cultivated on Trans well polycarbonate filters with a diameter of 6.5 mm and a pore size of 0.4 mm.18 The cells were seeded in a filter at a density of 0.15 106 cells/mL and cultured in Dulbecco's modified Eagle's media supplemented with 10% fetal calf serum, 1% nonessential amino acids, 100 U/mL penicillin, and 100 mg/mL streptomycin. The filters were removed and ready for proteomic analysis after 21 days in culture.

Analysis of Proteins Preparation of Samples

Filters that contained 21-day Caco-2 cultures were subjected to a 5-minute lysis at 100◦C in a solution that included 0.1 M Tris-HCl, pH 7.8, 2% sodium dodecyl sulfate, and 0.05 M dithiothreitol. Using the multi-enzyme digestion filter-aided sample preparation approach, which included Lys-C and trypsin, samples were processed in 30-kDa ultrafiltration units. To get two fractions per protease, 19 peptides were put onto pipet-tip strong anion exchange columns and eluted at pH 6 and pH 2, respectively.20 the tryptophan fluorescence assay was used to quantify the quantities of proteins and peptides.21

Analysis using Liquid Chromatography and Tandem Mass Spectrometry

In accordance with earlier instructions, the peptides were separated using a 3-hour acetonitrile gradient in 0.1% formic acid and then examined using a Q Enactive mass spectrometer (Thermo Fisher Scientific, Germany).22

Analyzing Data

The MaxQuant software, version 1.2.6.20, was used for the analysis of the mass spectrometric data. When looking for a protein, researchers used peptide MS and MS/MS data to search the UniProtKB/Swiss-Prot database, which had the most recent version from May 2013. The protein concentrations were determined by applying the total protein method to the raw spectral intensities of the MaxQuant output.24

Analysis using bioinformatics

Analyzing and Annotating Gene Ontologies combining the human GO annotation database file with the Ontologies software program, version 2.1.26, all of the proteome dataset was annotated using Gene Ontology (GO) concepts. On or around July 22, 2015. The GO Consortium's PANTHER classification system, version 10.0,27, was used to further categorize the selected proteins by protein class. Examination of Caco-Related Differentially Expressed Proteins For the purpose of identifying cells grown on filters or plastic proteins with significantly different quantities in the sample groups, a two-sample t-test was used, as implemented in Perseus, version 1.5.0.15. After doing 250 random permutations, a false discovery rate of 0.05 was used to determine statistical significance. The Ingenuity Pathway Analysis (IPA) program, version 24390178 (Qiagen, Hilden, Germany), which provides information on changed molecular and cellular processes, was used to examine significantly different proteins. Evidence derived only from experiments was considered in the study. Evaluation of the Relationships between Protein and Messenger RNA Expression via Statistical Means to enable comparison of datasets for distinct sets of transporters, the protein concentrations from this research and the messenger RNA (mRNA) expression levels from three prior studies28–30 were ranked and then adjusted to a scale from 0 to 1. Next, the Spearman rank correlation coefficient (rs) was used to connect the rankings of average normalized mRNA expression with the ranks of normalized protein concentration.

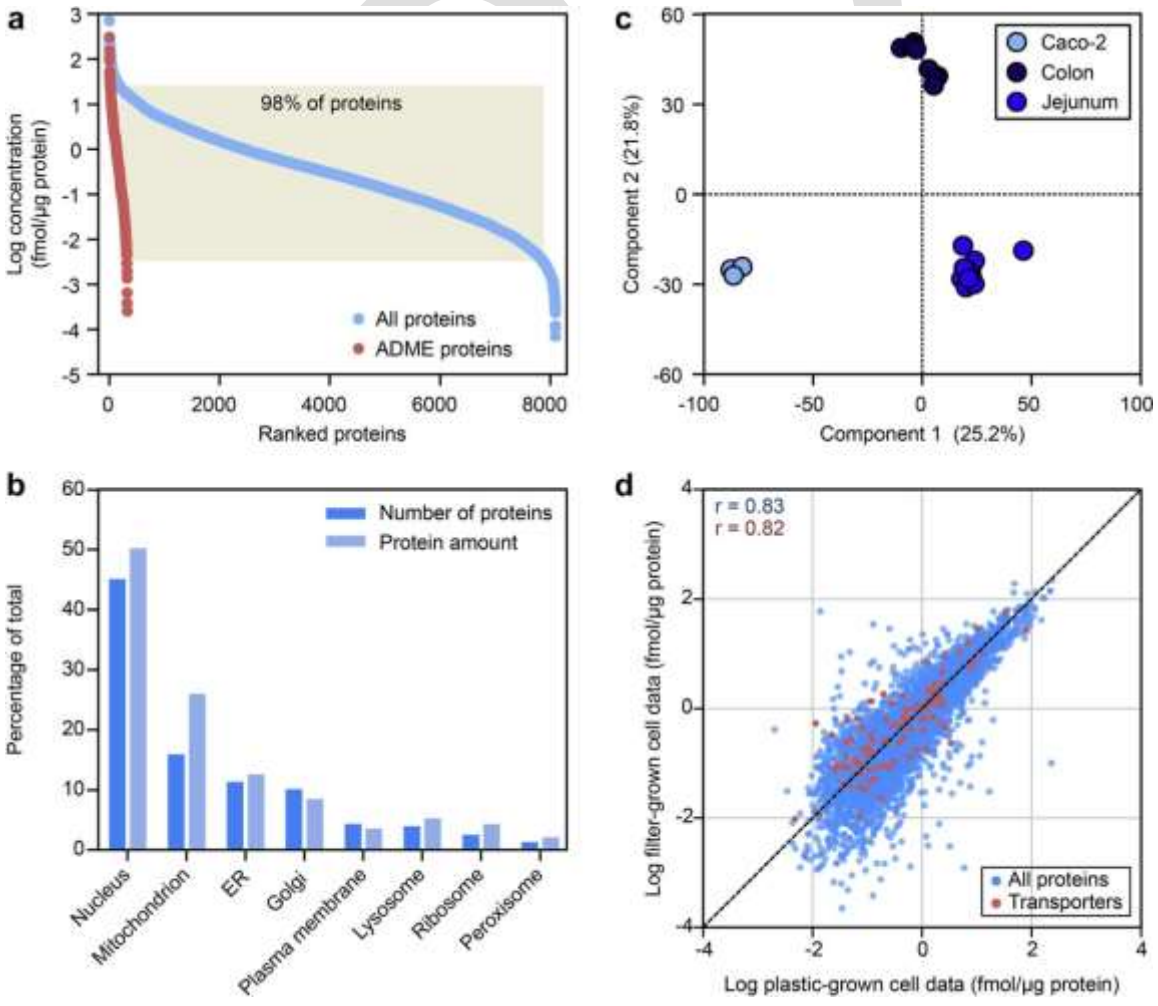
Transportation Trials

Following the procedure outlined earlier, Caco-2 cells (passage 98) were grown on 24-well plates. Prior to equilibrating for 10 minutes at 37◦C, the cells were washed three times with Hank's balanced salt solution, which has a pH of 7.4. The wells were then supplemented with new buffer that included pitavastatin (1-80 mm). The injection of ice-cold Hank's balanced salt solution halted transport after 4 minutes. Using 1 M NaOH, the cells were lysed. For every concentration of pitavastatin, cellular uptake was measured three times. Data from transportation trials were examined using a Waters Xevo mass spectrometer connected to an ACQUITY UPLC system (Waters, Milford, MA). The next step was to use a water-acetonitrile gradient that included 0.1% formic acid to separate them on a C-18 column (Waters). Results were measured with the use of Waters'Target Lynx program. We used the wells' protein concentrations to standardize the transport rates, and then we fitted them to a modified Michaelis-Menten equation with a linear diffusion factor, Pdiff.31

Analyzing the Regional Permeability of the Jejunum

simplified mechanistic model of intestinal permeability was developed in R version 3.1.1 (http://www.r-project.org) and used in conjunction with the deS-olve package for differential equation solution to simulate the jejunal permeability of pitavastatin.32 We used the ode function to execute the integration, which chooses an appropriate stiffness integration method automatically. The model had three parts: the intestines, the cells, and the blood. Its parameters were chosen to mimic the typical "Loc-I-Gut" perfusion experiment in humans, as well as the size of the small intestine seen in published studies.34 When it came to transporting compounds over the apical membrane, a mix of transporter-mediated uptake and bidirectional passive Fick's diffusion was used; on the other hand, flux across the basolateral membrane was thought to be entirely passive diffusion. Various other models that included MDR1/P-gp and BCRP-mediated efflux via the apical membrane and/or MRP3-mediated basolateral efflux were also considered.

Using cutting-edge protein quantification tools, we examined the global proteome of differentiated Caco-2 cell monolayers in this work. In keeping with standard practice for transport research, the cells were cultured on filter supports for a duration of three weeks. Our next step was to evaluate the worldwide Caco-2 protein



ADME-
relatedproteins.(b)Subcellularlocalizationoftheidentifiedproteins,classifiedbyGOanno
tations.(c)PCAofglobalproteomicdatafromCaco-
2cells,colon,andjejunum.Thetissuesampleswereclearlydistinguishablefromthe Caco-
2cells.Numbersinparenthesesshowhowmuchofthedatavariationeachcomponentexplai
ns.(d)Correlationofproteinconcentrationsin Caco-2 cells grown on filtersorplastic.
Pearsoncorrelationcoefficients(*r*)are given in theupperleftcorner. Transport proteins
arehighlightedin red.

explored. It was presumed that the mixing in the three sections would occur instantly. The usual drug residence period in the human jejunum is around 270 minutes, thus we mimicked the penetration process over that amount of time. See Transport Experiments for information on how the fitted Caco-2 transport data was used to get 35 kinetic parameters (Km and Pdiff) for the OATP2B1-mediated flow. Using the relative concentration of transporter proteins in the two systems, the maximum transporter activity method was used to scale the Caco-2 data and determine the jejunal OATP2B1 Vmax.36 The quantities of proteins in the jejunum were tripled. This was done to make up for the fact that, according to microscopy of representative tissue slices, jejunal proteomics samples were diluted due to subepithelial tissue. We used hematoxylin-eosin to stain 5-mm slices of bone, fat, and paraffin-embedded human jejunal biopsies. From the subepithelial border of the preparation all the way around each villi, the ratio of epithelial to subepithelial cells was measured in a symmetrical fashion. The biopsies included, on average, one-third epithelial cells. Applying the maximum transporter activity method to data from membrane vesicle systems, the Vmax values of the MDR1, BCRP, and MRP3 efflux transporters were adjusted (Valdese et al., unpublished data, 2015). Following adjustment for jejunal transporter concentrations, the corresponding Vmax values used in the models

The Entire Proteome of Filter-Grown Caco-2 Cells in Differentiation

Quantitative global proteome analysis was carried out utilizing label-free mass spectrometry to provide a comprehensive understanding of the protein composition of filter-grown Caco-2 cells. There were 8096 proteins found, with concentrations ranging from 7.1 10—5 to 7.2 102 fmol/mg total protein, covering seven orders of magnitude. A staggering 98% of these samples had amounts that fell within a 4-logarithmic range (Fig. 1a). At least two peptides were able to identify almost all of the proteins (92.9%), whereas sixty-three percent were able to do so with six or more peptides. The majority of the protein mass, 95%, was made up of the 2400 most abundant proteins (Fig. S1).

The Place of Proteins Within Cells

The identified proteins were categorized by main subcellular classes using GO annotations (Fig. 1b). Forty-five percent, or half of the total protein quantity, was derived from nuclear sources. From the endoplasmic reticulum, 11% and 10%value of relevance for every biological activity. Proteins increased in filter-grown cells belonged mostly to metabolic pathways (including those involving carbohydrates, amino acids, and other macromolecules) and molecular transport.

Conversely, the majority of the proteins that were found to be increased in the plastic-grown cells were associated with activities related to gene expression and cell development (Table 1).

Membrane Proteins at the Brush Border

The presence of an apical brush boundary membrane, which houses several enzymes that hydrolyze peptide bonds, is a characteristic of differentiated intestinal epithelial cells.38,39 Violin (VIL1) is one of the 100 most highly expressed proteins (59 fmol/mg protein) and provides structural support for microvilli at the apical brush boundary. Figure 2a further confirms the presence of many enzymes known to localize near the brush boundary. Enterocyte differentiation markers sucrase-isomaltase and intracellular alkaline phosphatase were among those found. A completely differentiated Caco-2 phenotype was indicated by their high levels, which were 9.6 and 4.7 fmol/mg protein, respectively.2

The Proteins That Connect Adherents

Both endothelial and epithelial tissues have adherens junctions, which are significant intercellular protein complexes. The structure is connected to the actin cytoskeleton by means of cadherin adhesion receptors, which in turn are connected to a set of related cytoplasmic proteins, vinculin, and catenins.40 Specifically, E-cadherin and P-cadherin, also known as CDH-1 and CDH-3, were located here. Among the two, E-cadherin was more numerous by more than 100-fold (Fig. 2b), indicating its prevalence in epithelial tissue. Vinculin and all three of the catenin—p120, aE-, and b-catenin—were found in high amounts (CTNND1, CTNNA1, and CTNNB1). Additionally, three members of the nectin family, afadin, EPLIN, and PLEKHA7 (MLLT4, LIMA1, and PLEKHA7) were shown to be proteins involved with adherens junctions. As a result, Caco-2 cells had several essential adherens junction components.

Closed-Loop Proteins

The intestinal epithelial cells have tight connections that allow some ions and tiny polar solutes to pass through, creating a semipermeable paracellular barrier.41 Using hydrophilic integrity indicators like mannitol and polyethylene glycol, as well as transepithelial electrical resistance, Caco-2 cells create a paracellular barrier that is more robust than the small intestine in vivo and in vitro.42,43 dollars Paracellular barrier functions in the intestinal epithelium are carried out by the 27-member claudin family (CLDN) of junction proteins.44,45 In tight junctions, the claudins and other extracellular proteins like occluding (OCLN) make up the extracellular compartment. Intracellular scaffolding proteins like ZO-1 (TJP1) link the claudins to the cytoskeleton, which includes actin and microtubules. The local environment, including factors such as proinflammatory cytokines, bacterial toxins, and medicinal excipients, may affect many intracellular proteins because they contain phosphorylation sites. Therefore, the pharmaceutical sciences have shown a great deal of interest in tight junction modulation as a means to improve biopharmaceutical and medication absorption in the intestines.46

Different cell types have different distributions of the claudins, which are either barrier- or channel-forming. Fig. 2c shows that Caco-2 cells expressed the barrier-forming clau-dins claudin-1, -3, and -4. Claudin-2, -7, and -15 are claudins that have paracellular channel activities

and were identified in Caco-2 cells. It is worth noting that claudins 1 and 3, which create barriers, were much more common in

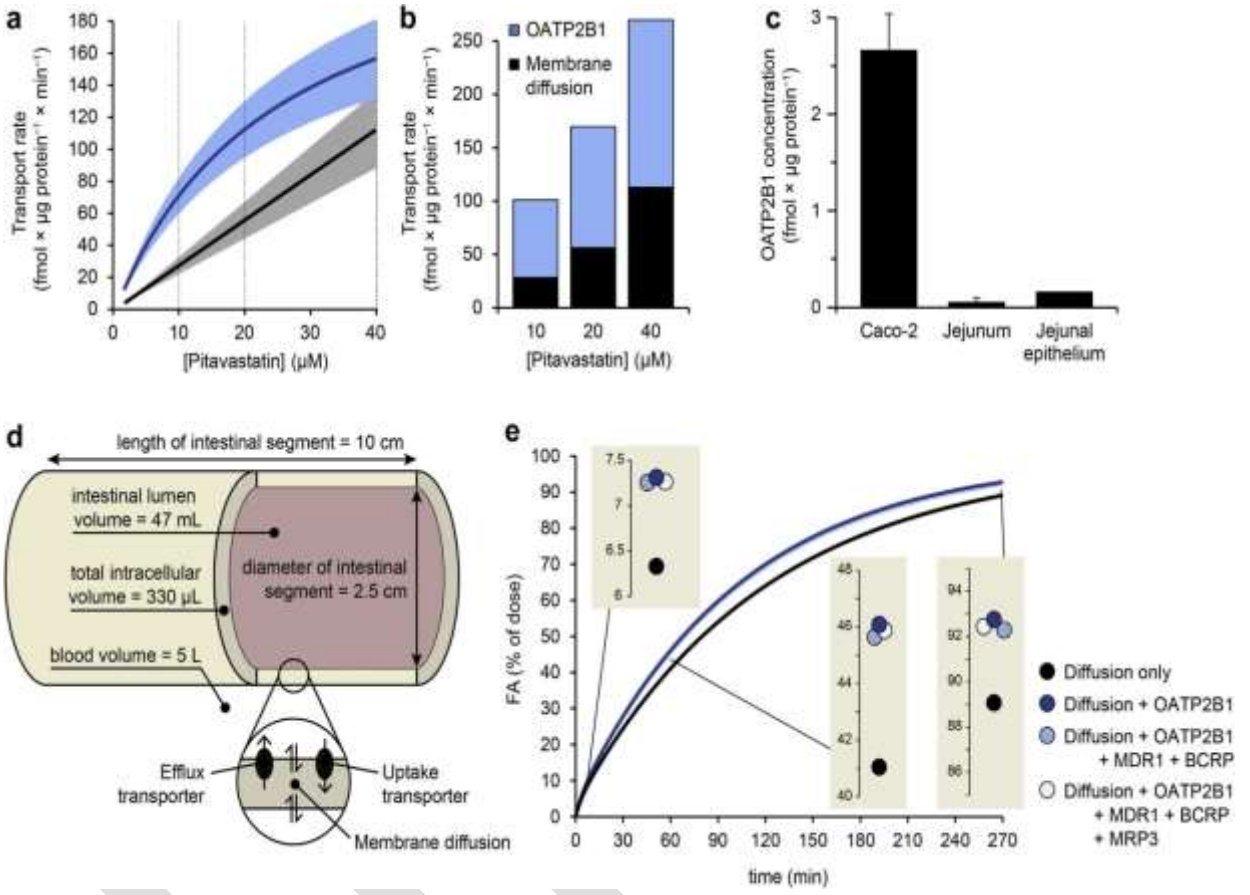


Figure 5. Transport of pitavastatin in Caco-2 cells and scaling to human jejunum. (a) Transport kinetics of pitavastatin in Caco-2 cells. The blue line indicates the saturableMichaelis-Mententransportcomponent, andtheblacklinethepassivediffusioncomponent. Shadedareasindicatestandarderrorsoftheparameterfitting. (b)ContributionfromOATP2B1 and passive membrane diffusion to the transport of pitavastatin, at concentrations expected in the small intestine after standard clinical doses (10, 20, and 40 mM,assuming dissolution of 1, 2, and 4 mg pitavastatin in 250 mL). (c) OATP2B1 protein concentrations in Caco-2 cells and human jejunal tissue. The rightmost bar shows the jejunalproteinconcentrationcorrectedforepithelial-to-subepithelialtissueintheanalyzedsamples.(d)Setupoftheintestinalpermeabilitymodel.Themodelwasconstructedtomimicatypical human intestinal perfusion experiment in a 10-cm intestinal segment and consisted of 3 compartments (intestine, intracellular, and blood). Intercompartment drugtransport was implemented as bidirectional passive diffusion and/or transporter-mediated flux across the apical and basolateral membranes. (e) Simulation of pitavastatin ab-
sorptionovertime,basedonkineticparametersandjejunaltransporterconcentrations.Blacklineandpointsindicateresultsfromsimulationswithonlypassivetransmembrane

diffusion included in the model. Blue lines and points indicate results from models that in addition to passive membrane diffusion also incorporated transporter-mediated
flux:modelsincludingOATP2B1;OATP2B1,MDR1,andBCRP;orOATP2B1,MDR1,BCRP,andMRP3,areshowninincreasinglylighterbluecolor.Insetsshowthesimulatedabsorptionattime¼45,60,and270min.

To summarize ADME protein expression in Caco-2 cells, we selected a list of 683 genes associated to ADME as our starting point. Figure 1a shows that the complete dataset had a comparable concentration range to the filter-grown Caco-2 cells, which accounted for around half of these (327 proteins), or 4% of the total identified proteins. Fig. 3a shows the results of additional protein classification using the PANTHER technology. Hydrolases, oxidoreductases,

transporters, transferases, and nucleic acid binding proteins were the top protein classes in terms of number of proteins. Fig. 3b shows that clustering of samples was comparable when PCA was performed using the subset of ADME proteins identified across Caco-2 cells, colon, and jejunum, as compared to when the whole proteomes were utilized.

Proteins that carry cargo

Substrates are transported across cellular membranes by two significant protein families: the solute carrier (SLC) and the ATP-binding cassette (ABC) trans-porters. Minimum of 395 SLCs in humans

in addition to 49 ABC transporters.53,54 Figure 3c and S2 show that at least two peptides were able to identify a total of eleventh-two SLC and twenty ABC transporters in this region. The concentrations varied between 0.001 fmol/mg protein for SLC45A4 and 55 fmol/mg protein for ANT2 and SLC25A5. There were 56 transporters, most of which were found on the plasma membranes either at the top or the bottom of cells, and 53 that were within the cells themselves. This highlights the significance of the recent plea for further study on transport proteins, as the subcellular localization of the other 23 transport proteins is not well-annotated.55

Enzymes for Metabolism

Figure 3d shows that this location was home to a number of enzymes involved in the first and second stages of xenobiotic metabolism, including nine cytochrome P450 (CYP) enzymes, many glutathione S-transferases and sulfotransferases. Xanthine dehydrogenase had a concentration of 0.005 fmol/mg protein, whereas glutathione S-transferase A2 had a concentration of 306 fmol/mg protein. The absence of important intestine drug-metabolizing CYP enzymes, such CYP3A4, is in line with earlier results.56 to 58 The CYP27A1 enzyme, which is involved in the conversion of cholesterol to bile acids, was the most abundant.59 Colorectal cancer is associated with an upregulation of four CYP enzymes: CYP2S1, CYP2W1, CYP51A1, and CYP2U1.60,61

Drug Interaction and Pharmacokinetic Transporters

Currently, 37 proteins from various organs and tissues are on the list of transporters that the International Transporter Consortium considers to be therapeutically significant.62,63 pages Figure 4a shows that nine out of twelve transporters discovered in intestinal epithelia (OATP2B1, MCT1, PEPT1, ENT1, OSTa-OSTb, P-gp, MRP3, MRP2, and BCRP) were located here. But just one peptide could be used to identify the OSTa-OSTb b subunit.

Furthermore, MRP5, MRP6, and MRP1 were all identified. As an example, concentrations of MRP5 were 0.003, but OATP2B1 and P-gp were above 2 fmol/mg protein. Previous research using the same Caco-2 clone has also shown a significant concentration of OATP2B1 in the current dataset.28,64

Transportation Protein Concentrations in Relation to Published Literature

Evaluation in Relation to mRNA ExpressionUsing the same clone of the Caco-2 cell line as before, we compared the amounts of transport proteins with mRNA expression data from three experiments.pages 28–30 This dataset identified fourteen of the thirty-two transporters that were studied in the aforementioned investigations (Fig. 4b). A Spearman rank correlation coefficient (rs) of 0.62 ($p < 0.05$) was obtained after ranking and normalizing the amounts of protein and mRNA expression. Furthermore, we compared our protein concentrations to the mRNA expression levels of 89 genes in Caco-2 cells cultivated in 10 separate labs.65 Figure S3 shows that out of the 76 genes identified in one lab, 29 were detected at the protein level, showing a similar connection as before (rs 0.61, $p < 0.001$). Consistent with other findings on the relationship between mRNA and protein levels in human cell lines, these figures are rather encouraging.66,67 peopleSubject for debateWe quantified the global proteome of mature Caco-2 cells grown on Trans well filters for 3 weeks using an in-depth, label-free mass spectrometry technique in this work. Therefore, we looked at the most used Caco-2 cell configuration for studying drug permeability. In order to concentrate the often sparsely expressed transporters, a combination of multi-enzyme digestion filter-aided sample preparation, total protein approach, state-of-the-art mass spectrometry, and the total protein method was used. This eliminated the need for a membrane preparation step. This meant that a major source of variation in earlier studies on transporter proteomics was no longer an issue.71 Nearly half of the human genome's protein-encoding genes were found among the more than 8,000 proteins that were discovered.72 For the first time, our results provide light on how Caco-2 cells are employed for drug transport investigations, namely on their protein expression.

global proteome data from filter-grown Caco-2 cells and principal component analysis

Markers of malignant and normal phenotypes helped to explain, at least in part, the observed cell-tissue separation in human intestine samples (colon and jejunum). Consistent with a previous work that used a smaller dataset (1000-2000 proteins) to compare relative protein expression in healthy colon mucosa with five colorectal cancer cell lines (including Caco-2), our findings support this approach.73 Caco-2 cells were also shown to be more comparable to jejunum than colon, according to our research. This provides further evidence that the Caco-2 cell line, while originally from the colon, differentiates into a phenotype similar to that of the small intestinal epithelium.2,3 These findings are not restricted to a subset of markers but rather provide a comprehensive picture of cellular physiology as a result of the extensive examination of protein expression.

Focusing on the phenotype of differentiated intestinal epithelial cell monolayers, we extracted concentration data for four specific protein groups—brush border proteins, integrins, adherens junctions, and tight junctions—to investigate the resolution at which information about these groups could be obtained. At these sites, almost every prototypic protein was located. To our knowledge, this is the best resolution attained so far in these cells; for instance, a huge number of tight junction proteins were identified and quantified.

From this inventory, what kind of data may be retrieved? To start with, the low permeability of tight junctions in Caco-2 monolayers was explained by the observed composition of claudins.42,43 dollars Secondly, key components for the control of tight junction perme-ability

are likely present because of the existence of many peripheral scaffolding proteins that connect the tight junctions to the cytoskeleton.from 74 to 77 Third, tricellulin is expressed at low levels, which means that the protein has been delivered to its final destination at the sites of contact between tricellular junctions. This shows that the cell monolayer is well-differentiated. We are hoping that researchers will utilize the global Caco-2 proteome in a similar way before doing studies on specific protein activities.

Compared to focused proteomic approaches, the global proteome technique used here allows for a deeper level of analysis. For instance, focused proteomics12 failed to find four of the thirteen clinically important transporters62,63, all of which are members of the ABCC/MRP family. These proteins were easily identified in our study. Proteins may be expressed at extremely low copy numbers that are below the detection threshold, therefore just because a protein isn't found in the proteome doesn't mean it isn't expressed. On the other hand, the data showing that proteins like CYP3A4, which are often found in large quantities in intestinal epithelia, are not present in this research, are more strongly conclusive. Additionally, a target protein's presence does not

Up for discussion

In this study, we used a comprehensive, label-free mass spectrometry method to quantify the global proteome of fully grown Caco-2 cells that were cultured on Trans well screens for three weeks. As a result, we examined the standard Caco-2 cell arrangement for investigating drug permeability. The often sparsely expressed transporters were concentrated using a combination of the total protein technique, multi-enzyme digestion filter-aided sample preparation, state-of-the-art mass spectrometry, and the total protein approach. As a result, preparing the membrane was superfluous. Consequently, a key variable that had plagued prior research on transporter proteomics was rendered moot.71 The discovery of over 8,000 proteins coincides with the location of about half of the genes in the human genome that encode proteins.72 Specifically, our findings provide insight on the protein expression of Caco-2 cells, which has been hitherto unseen in drug transport research.

results of the global proteome study using principal component analysis and filter-grown Caco-2 cells

The observed cell-tissue separation in human intestinal samples (colon and jejunum) was partially explained by markers of malignant and normal phenotypes. Our results corroborate those of a prior study that compared the relative expression of proteins in healthy colon mucosa and five colorectal cancer cell lines (including Caco-2) using a smaller dataset (1000-2000 proteins).From what we can see, 73 Caco-2 cells are more like jejunum cells than colon cells. This lends credence to the idea that the Caco-2 cell line, which originates in the colon, may undergo differentiation to resemble the small intestine epithelium.2,3 These results are not limited to a small group of markers but, thanks to the thorough analysis of protein expression, provide a complete picture of cellular physiology.

We investigated the resolution at which information about four specific protein groups—brush border proteins, integrin, adherens junctions, and tight junctions—by extracting concentration data from differentiated intestinal epithelial cell monolayers, with a focus on their phenotype.

These locations were shown to include almost all prototypic proteins. So far, this is the highest level of resolution that these cells have been able to achieve; for example, a large number of tight junction proteins were detected and measured.

What kinds of information may be gleaned from this inventory? To begin, the Caco-2 monolayers' low permeability of tight junctions was accounted for by the claudin composition that was found.42.4 dollars Secondly, there are several peripheral scaffolding proteins that link the cytoskeleton to the tight junctions, therefore it's probable that important components for controlling the permeability of these junctions are present.in the range of 74–77 The third finding is that tricellular junctions are the ultimate destinations of the protein since tricellulin expression is minimal. The highly differentiated cell monolayer is shown by this. In the future, we would love it if scientists would use the global Caco-2 proteome in a similar fashion before studying individual proteins' functions.

This study makes use of a global proteome methodology, which permits a more in-depth examination than targeted proteomic methods. One example is the ABCC/MRP family of transporters; targeted proteomics12 did not identify any of the thirteen transporters that are clinically significant62,63. We had no trouble identifying these proteins in our research. Just because a protein isn't included in the proteome doesn't imply it isn't expressed; proteins might be expressed at copy counts so low that they are undetectable. Data demonstrating the absence of proteins like CYP3A4, which are typically abundant in intestinal epithelia, are, however, more definitive. The presence of a target protein also

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Conflict of Interest

The Author declares that there is no conflict of interest.

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