Research Article

Coffee Polyphenols Change the Expression of STAT5B and ATF-2 Modifying Cyclin D1 Levels in Cancer Cells

Carlota Oleaga, 1 Carlos J. Ciudad, 1 Véronique Noé, 1 and Maria Izquierdo-Pulido 2

1 Department of Biochemistry and Molecular Biology, School of Pharmacy, University of Barcelona, 08028 Barcelona, Spain
2 Department of Nutrition and Food Science, School of Pharmacy, University of Barcelona, 08028 Barcelona, Spain

Correspondence should be addressed to Véronique Noé, vnoe@ub.edu

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Background. Epidemiological studies suggest that coffee consumption reduces the risk of cancer, but the molecular mechanisms of its chemopreventive effects remain unknown. Objective. To identify differentially expressed genes upon incubation of HT29 colon cancer cells with instant caffeinated coffee (ICC) or caffeic acid (CA) using whole-genome microarrays. Results. ICC incubation of HT29 cells caused the overexpression of 57 genes and the underexpression of 161, while CA incubation induced the overexpression of 12 genes and the underexpression of 32. Using Venn-Diagrams, we built a list of five overexpressed genes and twelve underexpressed genes in common between the two experimental conditions. This list was used to generate a biological association network in which STAT5B and ATF-2 appeared as highly interconnected nodes. STAT5B overexpression was confirmed at the mRNA and protein levels. For ATF-2, the changes in mRNA levels were confirmed for both ICC and CA, whereas the decrease in protein levels was only observed in CA-treated cells. The levels of cyclin D1, a target gene for both STAT5B and ATF-2, were downregulated by CA in colon cancer cells and by ICC and CA in breast cancer cells. Conclusions. Coffee polyphenols are able to affect cyclin D1 expression in cancer cells through the modulation of STAT5B and ATF-2.

1. Introduction

Polyphenols are the most abundant antioxidants in the diet. Their main dietary sources are fruits and plant-derived beverages such as fruit juices, tea, coffee, and red wine. Current evidence strongly supports a contribution of polyphenols to the prevention of cardiovascular diseases, cancers, and osteoporosis suggesting a role of these antioxidants in the prevention of neurodegenerative diseases and diabetes mellitus [1].

It is well established that polyphenol ingestion results in an increase of the plasma-antioxidant capacity. However, there is still some uncertainties about their efficiency to enhance the protection of cellular components, such as lipids or DNA, against oxidative stress in humans [2]. Polyphenols and other antioxidants were thought to protect cell constituents against oxidative damage by scavenging free radicals. However, this concept now appears to be an oversimplified view of their mode of action [3]. More likely, cells respond to polyphenols mainly through direct interactions with receptors or enzymes involved in signal transduction, which may result in modification of the redox status of the cell and may trigger a series of redox-dependent reactions [4]. This could also apply to the anticarcinogenic effects of polyphenols, which properties may be explained by many different mechanisms.

Hydroxycinnamic acids are a major class of polyphenols found in almost every plant [2]. The major representative of hydroxycinnamic acids is caffeic acid, which occurs in food mainly as an ester with quinic acid named chlorogenic acid (5-cafeoylquinic acid). Coffee is a major source of chlorogenic acid in the human diet; the daily intake in coffee drinkers is 0.5–1 g whereas coffee abstainers will usually ingest <100 mg/day. Studies have shown that approximately the 33% of ingested chlorogenic acid and the 95% of caffeic acid are absorbed intestinally [5]. Thus, about two-thirds of ingested chlorogenic acid reach the colon where it is probably metabolized to caffeic acid [6].

Bioavailability data suggest that the biological effects of chlorogenic acid would become apparent after its metabolism to caffeic acid, and hence the need of studying the effects of this acid. Chlorogenic acid and caffeic acid are
antioxidants in vitro [7], and they might inhibit the formation of mutagenic and carcinogenic N-nitroso compounds since they are inhibitors of the N-nitrosation reaction in vivo [8]. Furthermore, chlorogenic acid can inhibit DNA damage in vitro [9] as it inhibits lipid peroxidation-induced DNA adduct formation [10] and suppresses reactive oxygen species-mediated nuclear factor (NF-κB), activator protein-1 (AP-1), and mitogen-activated protein kinase activation by upregulating antioxidant enzymes [11]. These studies suggested that coffee polyphenols are potent chemopreventive agents.

Recent meta-analyses demonstrate inverse associations between coffee intake and the risk of colon, liver, breast, and endometrial cancer [12–15]. Moreover, in prospective population-based cohort studies, the inverse association between coffee consumption and risk of cancer has been shown. The group of Naganuma [16] found that the consumption of at least one cup of coffee per day was associated with a 49% lower risk of upper gastrointestinal cancer in a Japanese population, while Wilson and collaborators [17] found that men who regularly drink coffee appeared to have a lower risk of developing a lethal form of prostate cancer. The lower risk was evident when consuming either regular or decaffeinated coffee. It has been proposed that the inverse association between coffee intake and colon cancer could be explained, at least in part, by the presence of chlorogenic acid in coffee [18]. Ganmaa et al. [19] observed a general protective effect of caffeine intake on breast cancer risk for both ER subtypes, but the effect was only found to be significant for ER-positive breast cancers. In this study, the association between caffeine and breast cancer was stronger among postmenopausal women with estrogen-receptor and progesterone-receptor-positive breast cancer than those with estrogen-receptor and progesterone-receptor negative breast cancer [19]. In another study, coffee drinking specifically reduced the risk of developing ER-negative breast cancer but not ER-positive breast cancer [20].

Although there is enough evidence from epidemiological data supporting that coffee seems to reduce the risk of certain cancers, the molecular mechanisms underlying the chemopreventive effects of coffee remain unknown. For this reason, the aim of our study was to determine the effect at the molecular level of coffee polyphenols at low concentrations equivalent to one cup of coffee, using as a model a human colon cancer cell line HT29 in a nutrigenomic approach. Furthermore, the effect of coffee polyphenols was also evaluated in breast cancer cells.

2. Materials and Methods

2.1. Materials and Chemicals. Cells were incubated with Instant Caffeinated Coffee (ICC) (regular lyophilized instant coffee) and Caffeic acid (CA, Sigma). Compounds were dissolved either in DMSO (CA), or sterile water (ICC), and stored at −20°C.

2.2. Cell Culture. Colon adenocarcinoma HT29 and breast cancer MCF-7 cell lines were routinely grown in Ham's F12 medium supplemented with 7% fetal bovine serum (FBS, both from Gibco) at 37°C in a 5% CO₂ humidified atmosphere in 10 cm dish, or in 33 mm plate.

Cells were incubated with ICC or CA at concentrations equivalent to one cup of coffee. The concentrations used in cell incubations, 7 μg/mL in H₂O mQ for ICC and 1.68 μg/mL in DMSO for CA, respectively, took into account the amount of these compounds in one cup of coffee and their distribution in a regular human body with 75% water content. These concentrations did not cause any cytotoxic effect in the cell incubations as determined by the MTT assay [21].

2.3. Microarrays. Gene expression was analyzed by hybridization to The GeneChip Human Genome U133A plus 2.0 microarrays from Affymetrix, containing 47,000 transcripts and variants. HT29 cells were incubated with ICC and CA for 24 h. Total RNA was prepared from triplicate samples using SpeedTools Total RNA Extraction Kit (Biotools) following the recommendations of the manufacturer. RNA quality was tested by 2100 Bioanalyzer Eukaryote Total RNA Nano Series II (Agilent Technologies). Labeling, hybridization, and detection were carried out following the manufacturer’s specifications at the IDIBAPS Genomic Service (Hospital Clinic, Barcelona).

2.4. Microarray Data Analyses. Quantification was carried out with GeneSpring GX v.11.5.1 software (Agilent Technologies), which allows multfilter comparisons using data from different experiments to perform the normalization, generation of lists, and the functional classification of the differentially expressed genes. The input data was subjected to preprocess baseline transformation using the Robust Multiarray Average summarization algorithm using the median of control samples. After grouping the triplicate of each experimental condition, list of differentially expressed genes could be generated by using volcano plot analysis. The expression of each gene is reported as the ratio of the value obtained after each condition relative to control condition after normalization and statistical analysis of the data. The corrected P value cutoff applied was of <0.05; then the output of this statistical analysis was filtered by fold expression, selecting specifically those genes that had a differential expression of at least 1.3-fold. Gene classification was established by the Gene Ontology database.

2.5. Common Genes between ICC and CA Treatments. Common genes were selected from the lists of differentially expressed genes for each treatment using Venn-Diagrams. The newly generated list contained both over and underexpressed genes.

2.6. Generation of Biological Association Networks. BANs were constructed with the aid of the Pathway Analysis within the GeneSpring v.11.5.1 (Agilent) as described in Selga et al. [22] with the list of common genes differentially expressed in both treatments. A filtered screening was processed by the program between our data and bibliographic interaction.
databases up to a total of 100 related genes. Network associations were confirmed in the literature.

2.7. RT Real-Time PCR. Total RNA was extracted from HT29 cells using Ultraspec (Biotex) in accordance with the manufacturer’s instructions.

Complementary DNA was synthesized as described in Selga et al. [23] and the cDNA product was used for amplification by real time PCR. STAT5B and ATF-2 mRNA levels were determined in an ABI Prism 7000 Sequence Detection System (Applied Biosystems) using 3μL of the cDNA reaction and the assays-on-demand Hs00560035_m1 for STAT5B, Hs00153179_ml for ATF-2, and Hs00356991_m1 for APRT (all from Applied Biosystems). APRT mRNA was used as an endogenous control. The reaction was performed following the manufacturer’s recommendations. Fold changes in gene expression were calculated using the standard ΔΔCt method.

2.8. Western Blot. Whole extracts were obtained from 2.5 × 10⁶ control or treated cells according to Selga et al. [23]. Five μL of the extract was used to determine protein concentration by the Bradford assay (Bio-Rad). The extracts were frozen in liquid N2 and stored at −80°C. Total extracts (50μg) were resolved on SDS-polyacrylamide gels and transferred to PVDF membranes (Immobilon P, Millipore) using a semidyrid electroblotter.

The SNAP i.d. protein detection system technology (Millipore) was used to probe the membranes. This system applies vacuum through the membrane to actively drive reagents to protein locations, unlike the traditional technique of diffusion over the membrane as a reagent transport. Table 1 compiles the antibodies used in the different determinations.

Signals were detected by secondary horseradish peroxidase-conjugated antibody, either anti-rabbit (1:5000 or 1:10000 dilution; Dako) or anti-mouse (1:2500 dilution, Amersham NIF 824) and enhanced chemiluminescence using the ECL method, as recommended by the manufacturer (Amersham). Chemiluminescence was detected with ImageQuant LAS 4000 Mini technology (GE Healthcare).

2.9. Statistical Methods. For the RT-PCR and Western blot analyses, values are expressed as the mean ± SE of three different experiments. Data were evaluated by unpaired Student’s t test, and analyses were performed using the PASW Statistics v. 18.0.0. software.

3. Results

3.1. Effect of ICC and CA Incubations in HT29 Gene Expression. The expression profile of over 47,000 transcripts and variants included in the microarray HG U133 plus 2.0 from Affymetrix was compared between HT29 control cells and cells incubated with either CA or ICC, at nontoxic concentrations for 24 h. GeneSpring GX software v.11.5.1 was used to analyze the results. A list of differentially expressed genes by 1.3-fold with a P value cutoff of <0.05 was generated as described in Methods. When HT29 cells were incubated with ICC, 57 genes were overexpressed whereas 161 genes were underexpressed. Among the overexpressed genes, 24% belonged to the Transcription factors category and 19% to Cell cycle or to Biosynthetic processes. Within the underexpressed genes, the category corresponding to cell cycle was the most affected (53% of the genes) followed by Transcription factors (19%) and Biosynthetic processes (12%). Upon incubation with CA, 12 genes were overexpressed whereas 32 genes were underexpressed. Among the overexpressed genes, 33% belonged to the Transcription factors category, 25% to Cell cycle, and 16.7% to Biosynthetic processes or immune response. Within the underexpressed genes, again the category corresponding to Cell cycle was the most affected (30% of the genes) followed by Biosynthetic processes (15%) and Transcription factors (12%). The lists of differentially expressed genes are presented as Tables 2, 3, 4, and 5. The data presented in this work have been deposited in the Gene Expression Omnibus (GEO, http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO series accession number [GSM867162].

3.2. Generation of Biological Association Networks. A Biological Association Network (BAN) was constructed using the Pathway Analysis within GeneSpring v.11.5.1 as described in Methods using as the starting list the common genes differentially expressed upon incubation with CA and ICC. This list included five overexpressed genes and twelve underexpressed genes (Table 6). In the generated network, signal transducer and activator of transcription 5B (STAT5B) and activating transcription factor 2 (ATF-2) appeared as highly interconnected nodes (Figure 1). These two main nodes were selected for further validations. STAT5B was overexpressed with respect to the control by 23.8% in cells treated with ICC and by 33.4% in cells treated with CA, whereas ATF-2 was found underexpressed in HT29 incubated with ICC (32.5% decrease compared to the control) and with CA (26% decrease).

3.3. Validation of STAT5B and ATF-2 Changes at the mRNA and Protein Levels. STAT5B overexpression in HT29 cells upon incubation with CA and ICC was confirmed at the mRNA (1.16- and 1.3-fold compared to the control, respectively) and protein levels (1.5- and 1.2-fold compared to the control, respectively) (Figures 2(a) and 2(c)). In the case of ATF-2, the changes in mRNA levels were confirmed for both CA and ICC (0.88- and 0.86-fold compared to the control, respectively), whereas the decrease in protein levels was only observed in CA-treated cells (0.62-fold compared to the control) (Figures 2(b) and 2(d)).

3.4. Expression of Cyclin D1 upon Incubation with ICC and CA. Cyclin D1 is overexpressed at the mRNA and protein level in over 50% of the breast cancers either in the presence or absence of gene amplification, and it is one of the most commonly overexpressed proteins in breast cancer [24, 25]. Cyclin D1 transcription is regulated by STAT5 [26–29] and ATF-2 [30–32].
Figure 1: Biological association network (BAN) of differentially expressed genes in common between CA and ICC. The list of common genes between both treatments was used to construct a BAN with the Pathway Analysis software within GeneSpring v.11.5.1. An expanded network was constructed by setting an advanced filter that included the categories of binding, expression, metabolism, promoter binding, protein modification, and regulation. Only proteins are represented. The BAN shows the node genes STAT5B and ATF-2 that were further studied.

Table 1

| Antibody  | Molecular weight (KDa) | Dilution used | Supplier                          |
|-----------|------------------------|---------------|-----------------------------------|
| STAT5B    | 95                     | 1: 200        | sc-835, Santa Cruz Biotechnology Inc. |
| ATF-2     | 72                     | 1: 200        | sc-6233, Santa Cruz Biotechnology Inc. |
| Cyclin D1 | 38                     | 1: 200        | sc-8396, Santa Cruz Biotechnology Inc. |
| β-actin   | 42                     | 1: 200        | A2066, Sigma                      |
| Tubulin   | 60                     | 1: 100        | CP06, Calbiochem                  |

We analyzed the levels of cyclin D1 by western blot in MCF-7 and HT29 cells upon incubation with ICC and CA. As shown in Figure 3(a), incubation of MCF-7 cells with either CA and ICC led to a drastic decrease in the levels of cyclin D1 protein, together with an increase in the levels of STAT5B, but not to a decrease in the levels of ATF-2. In HT29 cells, incubation with CA did not affect cyclin D1 levels, whereas the presence of ICC led to an increase in cyclin D1 levels (b).

4. Discussion

In this work we analyzed the gene expression profile of human cancer cells treated with either ICC or CA. Caffeic acid was chosen since it is the main representative of hydroxycinnamic acids. Using microarrays we identified the differential expression of specific genes involved in several biological pathways. The changes in mRNA expression of two outlier genes, STAT5B and ATF-2, observed in the microarrays were confirmed by RT real-time PCR, and the changes in protein levels were also analyzed by Western blot. The selection of STAT5B and ATF-2 was made according to the results obtained in the construction of a biological association network. Finally, the modulation of cyclin D1, a target of STAT5B and ATF-2 transcription factors, upon incubation with coffee polyphenols was also established.

We show that ICC and the amount of CA of one cup of coffee are able to induce STAT5B mRNA and protein
Figure 2: Quantitation of mRNA and protein levels for STAT5B and ATF-2 in HT29 cells. The mRNA levels of STAT5B (a) and ATF-2 (b) were determined in control HT29 cells (empty bars) and cells treated with caffeic acid (CA, filled bars) and instant caffeinated coffee (ICC, grey bars) by RT real-time PCR as described in Methods. Results are expressed in fold changes compared to the control and are the mean ± SE of 3 different experiments. *P < 0.05 compared with the corresponding control. The protein levels of STAT5B (c) and ATF-2 (d) were determined in control HT29 cells (empty bars) and cells treated with caffeic acid (CA, filled bars) and instant caffeinated coffee (ICC, grey bars) by Western blot. Blots were reprobed with an antibody against β-actin or tubulin to normalize the results. Results represent the mean ± SE of 3 different experiments. *P < 0.05 and **P < 0.01 compared with the corresponding control.
Table 2: List of overexpressed genes in HT29 cells upon incubation with instant caffeinated coffee.

| Gene symbol | Gene title                                                                 | P value | FC absolute | Regulation |
|-------------|-----------------------------------------------------------------------------|---------|-------------|------------|
| CALM3       | Calmodulin 3 (phosphorylase kinase, delta)                                  | 0.016   | 1.3         | Up         |
| CDC42EP1    | CDC42 effector protein (Rho GTPase binding) 1                              | 0.027   | 1.3         | Up         |
| FOXN3       | Forkhead box N3                                                             | 0.022   | 1.3         | Up         |
| KIR2DL1     | Killer cell immunoglobulin-like receptor, two domains, long cytoplasmic tail, 1 | 0.023   | 1.3         | Up         |
| ORAI2       | ORAI calcium release-activated calcium modulator 2                           | 0.011   | 1.3         | Up         |
| RAPGEF1     | Rap guanine nucleotide exchange factor (GEF) 1                              | 0.022   | 1.3         | Up         |
| STH         | Saitohin                                                                   | 0.031   | 1.3         | Up         |
| SLC39A3     | Solute carrier family 39 (zinc transporter), member 3                       | 0.028   | 1.3         | Up         |
| ZNF397OS    | Zinc finger protein 397 opposite strand                                      | 0.024   | 1.3         | Up         |
| ZP4         | Zona pellucida glycoprotein 4                                               | 0.046   | 1.3         | Up         |
| FGFR1L       | Fibroblast growth factor receptor-like 1                                   | 0.035   | 1.31        | Up         |
| ITGA9       | Integrin, alpha 9                                                           | 0.002   | 1.31        | Up         |
| IRAK1       | Interleukin-1 receptor-associated kinase 1                                  | 0.038   | 1.31        | Up         |
| OBSL1       | Obscurin-like 1                                                             | 0.008   | 1.31        | Up         |
| ORAI2       | ORAI calcium release-activated calcium modulator 2                           | 0.011   | 1.3         | Up         |
| RPS17L4     | Ribosomal protein S17-like 4                                               | 0.026   | 1.31        | Up         |
| STAT5B      | Signal transducer and activator of transcription 5B                         | 0.007   | 1.31        | Up         |
| TRABD       | TraB domain containing                                                      | 0.043   | 1.31        | Up         |
| MYO9B       | Myosin IXB                                                                  | 0.041   | 1.32        | Up         |
| NME7        | Nonmetastatic cells 7, protein expressed in (nucleoside-diphosphate kinase) | 0.037   | 1.32        | Up         |
| RPS6KA4     | Ribosomal protein S6 kinase, 90 kDa, polypeptide 4                           | 0.014   | 1.32        | Up         |
| SIRPA       | Signal-regulatory protein alpha                                              | 0.019   | 1.32        | Up         |
| TBX20       | T-box 20                                                                    | 0.035   | 1.32        | Up         |
| TCF20       | Transcription factor 20 (AR1)                                               | 0.022   | 1.32        | Up         |
| ALDH3B1     | Aldehyde dehydrogenase 3 family, member B1                                 | 0.005   | 1.33        | Up         |
| BGN         | Biglycan                                                                    | 0.029   | 1.33        | Up         |
| GNB4        | Guanine nucleotide binding-protein (G protein), b-polypeptide 4              | 0.044   | 1.33        | Up         |
| IFNA17      | Interferon, alpha 17                                                        | 0.026   | 1.33        | Up         |
| KY          | Kyphoscoliosis peptidase                                                    | 0.013   | 1.33        | Up         |
| SCARF1      | Scavenger receptor class F, member 1                                        | 0.025   | 1.33        | Up         |
| SERPINB8    | Serpin peptidase inhibitor, clade B (ovalbumin), member 8                   | 0.01    | 1.33        | Up         |
| FST         | Follistatin                                                                 | 0.025   | 1.34        | Up         |
| MOGAT1      | Monoacylglycerol O-acyltransferase 1                                        | 0.009   | 1.34        | Up         |
| PPARGC1A    | Peroxisome proliferator-activated receptor gamma, coactivator 1 alpha       | 0.015   | 1.34        | Up         |
| SUCLG2      | Succinate-CoA ligase, GDP-forming, beta subunit                             | 0.011   | 1.34        | Up         |
| SULT1B1     | Sulfitotransferase family, cytosolic, 1B, member 1                           | 0.018   | 1.34        | Up         |
| TBX10       | T-box 10                                                                    | 0.011   | 1.34        | Up         |
| ZNF503      | Zinc finger protein 503                                                     | 0.022   | 1.34        | Up         |
| HBA1        | Hemoglobin, alpha 1                                                         | 0.04    | 1.35        | Up         |
| MEPE        | Matrix, extracellular phosphoglycoprotein with ASARM motif                  | 0.001   | 1.35        | Up         |
| PPP1CB      | Protein phosphatase 1, catalytic subunit, beta isoform                       | 0.03    | 1.35        | Up         |
| ARV1        | ARV1 homolog (S. cerevisiae)                                                | 0.011   | 1.36        | Up         |
| BCL3        | B-cell CLL/lymphoma 3                                                       | 0.034   | 1.36        | Up         |
| CTRC        | Chymotrypsin C (caldecrin)                                                  | 0.045   | 1.36        | Up         |
| EPOR        | Erythropoietin receptor                                                      | 0.008   | 1.37        | Up         |
| HMGA1       | High-mobility group AT-hook 1                                               | 0.039   | 1.37        | Up         |
| IL19        | Interleukin 19                                                              | 0.018   | 1.38        | Up         |
| ABCC12      | ATP-binding cassette, subfamily C (CFTR/MRP), member 12                      | 6.00E-04| 1.39        | Up         |
| RAI1        | Retinoic acid induced 1                                                     | 0.017   | 1.39        | Up         |
Table 2: Continued.

| Gene symbol | Gene title                                      | P value | FC absolute | Regulation |
|-------------|-------------------------------------------------|---------|-------------|------------|
| KLF5        | Kruppel-like factor 5 (intestinal)              | 0.028   | 1.4         | Up         |
| CBWD1       | COBW domain containing 1                        | 0.044   | 1.41        | Up         |
| ASAH3       | N-acylsphingosine amidohydrolase (alkaline ceramidase) 3 | 0.039   | 1.43        | Up         |
| ABHD14B     | Abhydrolase domain containing 14B               | 0.03    | 1.45        | Up         |
| TLN1        | Talin 1                                         | 0.049   | 1.45        | Up         |
| ARHGAP23    | Rho GTPase-activating protein 23                | 0.024   | 1.65        | Up         |
| HINT3       | Histidine triad nucleotide binding protein 3    | 0.002   | 1.77        | Up         |
| ARHGIDIA    | Rho GDP dissociation inhibitor (GDI) alpha      | 0.034   | 1.83        | Up         |
| CALR        | Calreticulin                                    | 0.007   | 1.93        | Up         |

The table shows the list of overexpressed genes by 1.3-fold with a P value < 0.05 obtained in cells treated with instant caffeinated coffee and includes the gene symbol for all genes, and their associated description. The ratio columns correspond to the absolute fold change in expression relative to the control group and the type of regulation (up: upregulation).

Figure 3: Expression of cyclin D1 upon incubation with ICC and CA in HT29 and MCF-7 cells. (a) Quantitation of STAT5b (empty bars), ATF-2 (filled bars), and cyclin D1 (grey bars) protein levels in MCF-7 cells. The protein levels were determined in control MCF-7 cells (CNT) and cells treated with caffeic acid (CA) and instant coffee (ICC) by Western blot. Blots were reprobed with an antibody against β-actin to normalize the results. Results represent the mean ± SE of 3 different experiments. *P < 0.05 and **P < 0.01 compared with the corresponding control. (b) Quantitation of STAT5b (empty bars), ATF-2 (filled bars), and cyclin D1 (grey bars) protein levels in HT29 cells. The protein levels were determined in control HT29 cells (CNT) and cells treated with caffeic acid (CA) and instant coffee (ICC) by Western blot. Blots were reprobed with an antibody against β-actin to normalize the results. Results represent the mean ± SE of 3 different experiments. *P < 0.05 and **P < 0.01 compared with the corresponding control.
### Table 3: List of underexpressed genes in HT29 cells upon incubation with instant coffee.

| Gene symbol | Gene title | P value | FC absolute | Regulation |
|-------------|------------|---------|-------------|------------|
| ACBD5       | Acyl-coenzyme A binding domain containing 5   | 0.017   | 1.3         | Down       |
| CXADR       | Coxackie virus and adenovirus receptor       | 0.015   | 1.3         | Down       |
| FANCD2      | Fanconi anemia, complementation group D2     | 0.047   | 1.3         | Down       |
| FRYL        | FRY-like                                      | 0.039   | 1.3         | Down       |
| NUB1        | Negative regulator of ubiquitin-like proteins 1 | 0.029   | 1.3         | Down       |
| PBRM1       | Polybromo 1                                   | 0.004   | 1.3         | Down       |
| PRKACB      | Protein kinase, cAMP-dependent, catalytic, beta | 0.033   | 1.3         | Down       |
| RIF1        | RAP1 interacting factor homolog (yeast)      | 0.012   | 1.3         | Down       |
| SLC39A6     | Solute carrier family 39 (zinc transporter), member 6 | 0.022   | 1.3         | Down       |
| TMEM170     | Transmembrane protein 170                    | 0.032   | 1.3         | Down       |
| WDR26       | WD repeat domain 26                          | 0.028   | 1.3         | Down       |
| RNGTT       | RNA guanyltransferase and 5'-phosphatase      | 0.04    | 1.3         | Down       |
| CTDSPL2     | CTD small phosphatase like 2                 | 0.03    | 1.3         | Down       |
| ZC3H11A     | Zinc finger CCCH-type containing 11A          | 0.014   | 1.3         | Down       |
| TMOD3       | Tropomodulin 3 (ubiquitous)                  | 0.0171  | 1.3         | Down       |
| CPD         | Carboxypeptidase D                           | 0.002   | 1.3         | Down       |
| CBL         | Cas-Br-M ecotropic retroviral transforming sequence | 0.008   | 1.3         | Down       |
| CDC42SE2    | CDC42 small effector 2                       | 0.022   | 1.3         | Down       |
| CLN5        | Ceroid-lipofuscinosis, neuronal 5            | 0.001   | 1.3         | Down       |
| DDX3X       | DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked | 0.027   | 1.3         | Down       |
| FGRF1OP2    | FGRF1 oncogene partner 2                     | 0.049   | 1.3         | Down       |
| LRRFIP1     | Leucine-rich repeat (in FLII) interacting protein 1 | 0.026   | 1.3         | Down       |
| PDCD4       | Programmed cell death 4                      | 0.005   | 1.3         | Down       |
| REPS2       | RALBP1-associated Eps domain containing 2     | 0.046   | 1.3         | Down       |
| SLC7A6      | Solute carrier family 7, member 6            | 0.002   | 1.3         | Down       |
| TFRC        | Transferrin receptor (p90, CD71)             | 0.038   | 1.3         | Down       |
| TMEM19      | Transmembrane protein 19                     | 0.024   | 1.3         | Down       |
| AGPS        | Alkylglycerone phosphate synthase             | 0.001   | 1.3         | Down       |
| SLCA47      | Solute carrier family 4, member 7            | 0.028   | 1.3         | Down       |
| SPTAN1      | Spectrin, alpha, nonerythrocytic 1 (alpha-fodrin) | 0.02    | 1.3         | Down       |
| GPD2        | Glycerol-3-phosphate dehydrogenase 2 (mitochondrial) | 0.033   | 1.3         | Down       |
| BICD1       | Bicaudal D homolog 1 (Drosophila)            | 0.008   | 1.3         | Down       |
| FBXW11      | F-box and WD repeat domain containing 11      | 0.025   | 1.3         | Down       |
| BCLAF1      | BCL2-associated transcription factor 1        | 0.025   | 1.3         | Down       |
| CDH1        | Cadherin 1, type 1, E-cadherin (epithelial)   | 0.011   | 1.3         | Down       |
| CLK4        | CDC-like kinase 4                            | 0.049   | 1.3         | Down       |
| PTAR1       | Protein prenyltransferase alpha subunit repeat containing 1 | 0.027   | 1.3         | Down       |
| SMEK2       | SMEK homolog 2, suppressor of mek1 (Dictyostelium) | 0.012   | 1.3         | Down       |
| CEPT1       | Choline/ethanolamine phosphotransferase 1     | 0.038   | 1.3         | Down       |
| SAR1A       | SAR1 gene homolog A (S. cerevisiae)           | 0.033   | 1.3         | Down       |
| PDGFc       | Platelet-derived growth factor C             | 0.02    | 1.3         | Down       |
| NEAT5       | Nuclear factor of activated T-cells 5, toxicity responsive | 0.045   | 1.3         | Down       |
| FRS2        | Fibroblast growth factor receptor substrate 2 | 0.03    | 1.3         | Down       |
| BMS1P5      | BMS1 pseudogene 5                            | 0.036   | 1.3         | Down       |
| GLS         | Glutaminase                                  | 5.00E-04| 1.3         | Down       |
| LMAN1       | Lectin, mannose binding, 1                   | 7.00E-04| 1.3         | Down       |
| ARHGAP18    | Rho GTPase-activating protein 18             | 8.00E-04| 1.3         | Down       |
Table 3: Continued.

| Gene symbol | Gene title                                                        | P value | FC absolute | Regulation |
|-------------|-------------------------------------------------------------------|---------|-------------|------------|
| ARHGAP5    | Rho GTPase-activating protein 5                                   | 0.006   | 1.33        | Down       |
| CCNE2       | Cyclin E2                                                         | 0.036   | 1.33        | Down       |
| SPCS3       | Signal peptidase complex subunit 3 homolog (S. cerevisiae)        | 0.008   | 1.33        | Down       |
| NCOA2       | Nuclear receptor coactivator 2                                    | 0.005   | 1.33        | Down       |
| SRPRB       | Signal recognition particle receptor, B subunit                   | 0.018   | 1.33        | Down       |
| TLK1        | Tousled-like kinase 1                                              | 0.04    | 1.33        | Down       |
| NCOA3       | Nuclear receptor coactivator 3                                    | 0.048   | 1.33        | Down       |
| STRN3       | Striatin, calmodulin-binding protein 3                            | 2.00E-04| 1.33        | Down       |
| AP1G1       | Adaptor-related protein complex 1, gamma 1 subunit                | 0.004   | 1.34        | Down       |
| B3GALNT2    | Beta-1,3-N-acetylgalactosaminyltransferase 2                       | 0.034   | 1.34        | Down       |
| PPHLN1      | Periphilin 1                                                      | 2.00E-04| 1.34        | Down       |
| SNX13       | Sorting nexin 12                                                  | 0.001   | 1.34        | Down       |
| TMED2       | Transmembrane emp24 domain-trafficking protein 2                  | 0.041   | 1.34        | Down       |
| BRWD1       | Bromodomain and WD repeat domain containing 1                    | 0.011   | 1.34        | Down       |
| HLA         | Major histocompatibility complex, class I, B                      | 0.028   | 1.34        | Down       |
| CHP         | Calcium-binding protein P22                                        | 0.002   | 1.34        | Down       |
| MTMR9       | Myotubularin-related protein 9                                    | 0.026   | 1.34        | Down       |
| DCUN1D4     | DLong1, defective in cullin neddylation 1, domain containing 4     | 0.031   | 1.34        | Down       |
| ARL6IP2     | ADP-ribosylation factor-like 6 interacting protein 2              | 0.02    | 1.35        | Down       |
| GLIS3       | GLIS family zinc finger 3                                          | 0.01    | 1.35        | Down       |
| LARP4       | La ribonucleoprotein domain family, member 4                       | 0.019   | 1.35        | Down       |
| PTPLB       | Protein tyrosine phosphatase-like member b                         | 0.036   | 1.35        | Down       |
| TRAM1       | Translocation-associated membrane protein 1                       | 0.002   | 1.35        | Down       |
| TMEM64      | Transmembrane protein 64                                           | 0.001   | 1.35        | Down       |
| CBFB        | Core-binding factor, beta subunit                                 | 0.005   | 1.35        | Down       |
| SELT        | Selenoprotein 12                                                  | 0.002   | 1.35        | Down       |
| PEX13       | Peroxisome biogenesis factor 12                                    | 0.011   | 1.35        | Down       |
| TNKS2       | TRF1-interacting ankyrin-related ADP-ribose polymerase 2           | 0.034   | 1.35        | Down       |
| TMPO        | Thymopoietin                                                      | 0.001   | 1.35        | Down       |
| LIN7C       | Lin-7 homolog C (C. elegans)                                       | 0.007   | 1.35        | Down       |
| MTA2        | Metastasis-associated 1 family, member 2                           | 0.013   | 1.36        | Down       |
| TMEM168     | Transmembrane protein 168                                          | 0.035   | 1.36        | Down       |
| CREBFZF     | CREB/ATF bZIP transcription factor                                 | 0.016   | 1.36        | Down       |
| OSTF1       | Osteoclast-stimulating factor 1                                    | 0.002   | 1.36        | Down       |
| WDR57       | WD repeat domain 57 (US snRNP specific)                            | 0.001   | 1.36        | Down       |
| GLT2D1      | Glycosyltransferase 25 domain containing 1                         | 0.008   | 1.36        | Down       |
| NAPG        | N-ethylmaleimide-sensitive factor attachment protein, gamma       | 0.015   | 1.36        | Down       |
| CCDC126     | Coiled-coil domain containing 12                                   | 0.039   | 1.37        | Down       |
| LASS6       | LAG1 homolog, ceramide synthase 6                                  | 0.005   | 1.37        | Down       |
| MYSM1       | Myb-like, SWIRM and MPN domains 1                                 | 0.021   | 1.37        | Down       |
| CYP51A1     | Cytochrome P450, family 51, subfamily A, polypeptide 1             | 0.007   | 1.37        | Down       |
| PDE4DIP     | Phosphodiesterase 4D interacting protein (myogalmin)               | 0.024   | 1.37        | Down       |
| SAP30L      | SAP30-like                                                        | 0.012   | 1.37        | Down       |
| PTPRJ       | Protein tyrosine phosphatase, receptor type, J                     | 0.011   | 1.37        | Down       |
| PGGT1B      | Protein geranylgeranyltransferase type I, beta subunit             | 9.00E-04| 1.37        | Down       |
| ASPH        | Aspartate beta-hydroxylase                                         | 0.011   | 1.37        | Down       |
| SEMA3C      | Sema domain, (semaphorin) 3C                                       | 0.036   | 1.38        | Down       |
| WDR76       | WD repeat domain 76                                                | 0.016   | 1.38        | Down       |
| Gene symbol | Gene title                                      | P value | FC absolute | Regulation |
|-------------|------------------------------------------------|---------|-------------|------------|
| ATP13A3     | ATPase-type 13A3                               | 0.002   | 1.38        | Down       |
| LMBR1       | Limb region 1 homolog (mouse)                  | 0.014   | 1.38        | Down       |
| GLUD1       | Glutamate dehydrogenase 1                      | 0.001   | 1.39        | Down       |
| GSTCD       | Glutathione S-transferase, C-terminal domain containing | 0.029   | 1.39        | Down       |
| SPTLC1      | Serine palmitoyltransferase, subunit 1          | 0.02    | 1.39        | Down       |
| U2AF1       | U2 small nuclear RNA auxiliary factor 1         | 9.00E-04| 1.39        | Down       |
| UHMK1       | U2AF homology motif (UHM) kinase 1             | 0.007   | 1.39        | Down       |
| ARGLU1      | Arginine and glutamate-rich 1                   | 6.00E-04| 1.39        | Down       |
| ANKRD12     | Ankyrin repeat domain 12                       | 0.03    | 1.39        | Down       |
| PPP3R1      | Protein phosphatase 3, regulatory subunit B, alpha isoform | 0.023   | 1.39        | Down       |
| XRN1        | 5'→3' exoribonuclease 1                        | 0.019   | 1.4         | Down       |
| CLSPN       | Claspin homolog (*Xenopus laevis*)             | 0.013   | 1.4         | Down       |
| CXADRPI     | Coxsackie virus and adenovirus receptor pseudogene 1 | 0.034   | 1.4         | Down       |
| G3BP1       | GTPase-activating protein- (SH3 domain) binding protein 1 | 0.002   | 1.4         | Down       |
| TMEM30A     | Transmembrane protein 30A                      | 0.01    | 1.4         | Down       |
| CLCN3       | Chloride channel 3                             | 0.035   | 1.41        | Down       |
| STK4        | Serine/threonine kinase 4                      | 0.039   | 1.41        | Down       |
| ZNF644      | Zinc finger protein 644                        | 0.02    | 1.41        | Down       |
| TCP11L1     | T-complex 11 (mouse)-like 1                    | 0.014   | 1.41        | Down       |
| SFRS6       | Splicing factor, arginine/serine-rich 6         | 0.031   | 1.41        | Down       |
| NPL         | N-acetylneuraminic pyruvate lyase              | 0.006   | 1.41        | Down       |
| G3BP2       | GTPase-activating protein- (SH3 domain) binding protein 2 | 0.001   | 1.42        | Down       |
| HNRNPU      | Heterogeneous nuclear ribonucleoprotein U       | 0.01    | 1.42        | Down       |
| TBL1XR1     | Transducin (beta)-like 1 X-linked receptor 1    | 0.001   | 1.42        | Down       |
| PHTF2       | Putative homeodomain transcription factor 2     | 0.002   | 1.42        | Down       |
| ADAM10      | ADAM metalloproteinase domain 10               | 0.011   | 1.43        | Down       |
| ADAM9       | ADAM metalloproteinase domain 9 (meltrin gamma) | 0.01    | 1.43        | Down       |
| MALAT1      | Metastasis-associated lung adenocarcinoma transcript 1 | 0.04    | 1.43        | Down       |
| SCARB2      | Scavenger receptor class B, member 2            | 0.001   | 1.43        | Down       |
| CANX        | Calnexin                                       | 0.043   | 1.43        | Down       |
| CASP2       | Caspase 2, apoptosis-related cysteine peptidase | 0.033   | 1.43        | Down       |
| TRPS1       | Trichorhinophalangeal syndrome 1                | 0.005   | 1.44        | Down       |
| ZFX         | Zinc finger protein, X-linked                  | 0.033   | 1.44        | Down       |
| SGPL1       | Sphingosine-1-phosphate lyase                  | 0.04    | 1.44        | Down       |
| PTPN11      | Protein tyrosine phosphatase, nonreceptor type 11 | 0.045   | 1.44        | Down       |
| SFRS11      | Splicing factor, arginine/serine-rich 11        | 0.045   | 1.45        | Down       |
| B3GNT5      | Beta-1,3-N-acetylglucosaminyltransferase 5      | 0.021   | 1.45        | Down       |
| MAP3K1      | Mitogen-activated protein kinase kinase kinase 1 | 0.019   | 1.45        | Down       |
| SNHG4       | Small nucleolar RNA host gene (nonprotein coding) 4 | 0.004   | 1.46        | Down       |
| PARD6B      | Par-6 partitioning defective 6 homolog beta (*C. elegans*) | 0.04    | 1.46        | Down       |
| ROD1        | ROD1 regulator of differentiation 1 (*S. pombe*) | 0.001   | 1.46        | Down       |
| SPTBN1      | Spectrin, beta, nonerythrocytic 1               | 0.02    | 1.48        | Down       |
| TXNDC1      | Thioredoxin domain containing 1                 | 0.013   | 1.48        | Down       |
| ATF2        | Activating transcription factor 2               | 0.005   | 1.48        | Down       |
| RDX         | Radixin                                        | 0.043   | 1.48        | Down       |
| SCAMP1      | Secretory carrier membrane protein 1           | 0.009   | 1.48        | Down       |
| PTAR1       | Protein prenyltransferase alpha subunit repeat containing 1 | 0.018   | 1.49        | Down       |
| RC3H2       | Ring finger and CCCH-type zinc finger domains 2 | 0.0037  | 1.49        | Down       |
Table 3: Continued.

| Gene symbol | Gene title                                      | P value | FC absolute | Regulation |
|-------------|------------------------------------------------|---------|-------------|------------|
| ADAM17      | ADAM metallopeptidase domain 17                | 0.007   | 1.49        | Down       |
| FAM76B      | Family with sequence similarity 76, member B   | 0.014   | 1.5         | Down       |
| ITGB8       | Integrin, beta 8                               | 1.00E-04| 1.5         | Down       |
| TRIM23      | Tripartite motif-containing 23                  | 0.005   | 1.5         | Down       |
| CASC5       | Cancer susceptibility candidate 5              | 0.019   | 1.52        | Down       |
| SLC16A1     | Solute carrier family 16, member 1             | 0.002   | 1.52        | Down       |
| FNBP1       | Formin-binding protein 1                        | 0.037   | 1.53        | Down       |
| PRKAR1A     | Protein kinase, cAMP-dependent, regulatory, type I, alpha | 9.00E-04 | 1.53        | Down       |
| B4GALT1     | Beta 1,4-galactosyltransferase, polypeptide 1   | 0.035   | 1.55        | Down       |
| MDM4        | Mdm4 p53-binding protein homolog (mouse)       | 0.011   | 1.58        | Down       |
| FGD4        | FYVE, RhoGEF, and PH domain containing 4        | 0.001   | 1.59        | Down       |
| UBA6        | Ubiquitin-like modifier activating enzyme 6     | 8.00E-04| 1.62        | Down       |
| ZDHHC21     | Zinc finger, DHHC-type-containing 21            | 0.036   | 1.64        | Down       |
| REEP3       | Receptor accessory protein 3                    | 7.00E-04| 1.65        | Down       |
| SSR3        | Signal sequence receptor, gamma                | 0.014   | 1.65        | Down       |
| ZDHHC20     | Zinc finger, DHHC-type-containing 20            | 0.003   | 1.66        | Down       |
| EIF2S3      | Eukaryotic translation initiation factor 2, subunit 3 gamma | 0.001   | 1.7         | Down       |
| HNRNPH1     | Heterogeneous nuclear ribonucleoprotein H1      | 0.011   | 1.79        | Down       |
| ATL3        | Atlastin 3                                      | 0.001   | 2.02        | Down       |

The table shows the list of underexpressed genes by 1.3-fold with a P value < 0.05 obtained in cells treated with instant caffeinated coffee and includes the gene symbol for all genes, and their associated description. The ratio columns correspond to the absolute fold change in expression relative to the control group and the type of regulation (down: downregulation).

Table 4: List of overexpressed genes in HT29 cells upon incubation with caffeic acid.

| Gene symbol | Gene title                                      | P value | FC absolute | Regulation |
|-------------|------------------------------------------------|---------|-------------|------------|
| SULT1B1     | Sulfotransferase family, cytosolic, 1B, member 1| 0.02    | 1.3         | Up         |
| BCL6B       | B-cell CLL/lymphoma 6, member B (zinc finger protein) | 3.00E-04 | 1.3         | Up         |
| KCNJ5       | Potassium inwardly-rectifying channel, subfamily J, member 5 | 0.01   | 1.31        | Up         |
| EPOR        | Erythropoietin receptor                          | 0.02    | 1.32        | Up         |
| DNAJC21     | Dnaj (Hsp40) homolog, subfamily C, member 21   | 0.049   | 1.33        | Up         |
| STAT5B      | Signal transducer and activator of transcription 5B | 0.012  | 1.33        | Up         |
| FST         | Follistatin                                      | 0.021   | 1.37        | Up         |
| CD84        | CD84 molecule                                    | 0.033   | 1.37        | Up         |
| THRA        | Thyroid hormone receptor, alpha                 | 0.017   | 1.37        | Up         |
| MAPK8IP3    | Mitogen-activated protein kinase 8 interacting protein 3 | 0.028  | 1.4         | Up         |
| SIAE        | Sialic acid acetylerase                          | 0.01    | 2.42        | Up         |
| HINT3       | Histidine triad nucleotide-binding protein 3    | 0.033   | 2.6         | Up         |

The table shows the list of overexpressed genes by 1.3-fold with a P value < 0.05 obtained in cells treated with caffeic acid and includes the gene symbol for all genes, their associated description. The ratio columns correspond to the absolute fold change in expression relative to the control group and the type of regulation (up: upregulation).

levels in HT29 cells. STAT5 was originally described as a prolactin-induced mammary gland factor [33]. The cloning of two closely related STAT5 CDAs, from both mouse and human CDNA libraries, showed two distinct genes, STAT5A and STAT5B that encoded two STAT5 proteins [34–37]. In addition to prolactin, STAT5 proteins are activated by a wide variety of cytokines and growth factors, including IL-2, IL-3, IL-5, IL-7, IL-9, IL-15, granulocyte-macrophage colony-stimulating factor, erythropoietin, growth hormone, thrombopoietin, epidermal growth factor, and platelet-derived growth factor. The key function of STAT5B is to mediate the effects of growth hormone [38, 39]. Modulation of STAT5 levels or transcriptional activity has already been described in cells treated with natural compounds such as nobiletin, a citrus flavonoid [40], thea flavins [41], and silibinin, a natural polyphenolic flavonoid which is a major bioactive component of silymarin isolated from Silybum marianum [42]. Furthermore, it has been reported that
The table shows the list of underexpressed genes by 1.3-fold with a P value < 0.05 obtained in cells treated with caffeic acid and includes the gene symbol for all genes, their associated description. The ratio columns correspond to the absolute fold change in expression relative to the control group and the type of regulation (down: downregulation).

butein, the major biologically active polyphenolic component of the stems of *Rhus verniciflua*, downregulated the expression of STAT3-regulated gene products such as Bcl-xL, Bcl-2, cyclin D1, and Mcl-1 [43].

STAT5B participates in diverse biological processes, such as growth development, immunoregulation, apoptosis, reproduction, prolactin pathway, and lipid metabolism. STAT5B deficiency is a recently identified disease entity that involves both severe growth hormone-resistant growth failure and severe immunodeficiency [44–46]. The induction of STAT5B expression upon incubation with CA and ICC could represent a nutritional tool to upregulate this transcription factor and suggests novel research strategies for natural therapies in Crohn's disease and inflammatory bowel disease in which STAT5B appears to maintain the mucosal barrier integrity and tolerance [47, 48]. In colorectal cancer both STAT5a and STAT5b play important roles in progression and downregulation of both STAT5A and STAT5B results in a gradual decrease in cell viability, predominantly attributed to G1 cell cycle arrest, and apoptotic cell death [49]. In this context the increase in STAT5B caused by ICC and CA would have a negative effect on colorectal cancer patients, as it would trigger cell proliferation and survival.

In human breast cancer, STAT5A/B has been shown a dual role in the mammary gland as an initiator of tumor formation as well as a promoter of differentiation of established tumors. STAT3, STAT5A, and STAT5B are overexpressed or constitutively activated in breast cancer [50–52] and active STAT5A/B in human breast cancer predicted favorable clinical outcome [53]. Prolactin receptor signal
transduction through the Jak2-STAT5 pathway has been considered to be essential for proliferation and differentiation of normal mammary epithelial cells [54-56]. It has been shown that the levels of NUC-pYSTAT5 decreased as breast cancer progressed from normal to in situ, to invasive, and then to nodal metastases [57]. Additionally Peck et al. [57] found that the absence of detectable NUC-pYStat5 in tumors of patients how where under antiestrogen therapy was associated with poor breast cancer-specific survival. We analyzed STAT5B modulation through the PRL pathway in MCF-7 cells. This differential behavior could be due to other ICC components besides CA. In this direction Rubach et al. [64] reported a different response in ATF-2 activity after incubation of a gastric cell line with different coffee compounds. The presence of pyrogallol, catechol, βN-alkanoylhydroxytryptamides, and N-methylpyridinium increased ATF-2 activity, whereas chlorogenic acid and caffeic acid decreased it [64]. In our conditions incubation of HT29 cells with ICC caused a modest decrease in ATF-2 mRNA levels. However this effect was not translated at the protein level. We hypothesize that ICC contains other polyphenols in addition to caffeic acid that are able to increase ATF-2 protein levels through an increase of the translation of its mRNA, the increase of stability of the protein or an inhibition of its degradation. In this direction several plant polyphenols such as (-)-epigallocatechins-3-gallate (EGCG), genistein, luteolin, apigenin, chrysin, quercetin, curcumin, and tannic acid have been described to possess proteasome-inhibitory activity [65, 66].

The downregulation of ATF-2 expression after CA and ICC incubation in HT29 cells reported here is in accordance with the observed decrease in activity of ATF-2 in gastric cells when incubating with chlorogenic acid, the precursor of caffeic acid [64]. Surprisingly, the validation of the protein levels showed the upregulation of ATF-2 protein with ICC, but not with CA, both in HT29 and MCF-7 cells. This differential behavior could be due to other ICC components besides CA. In this direction Rubach et al. [64] reported a different response in ATF-2 activity after incubation of a gastric cell line with different coffee compounds. The presence of pyrogallol, catechol, βN-alkanoylhydroxytryptamides, and N-methylpyridinium increased ATF-2 activity, whereas chlorogenic acid and caffeic acid decrease it [64]. In our conditions incubation of HT29 cells with ICC caused a modest decrease in ATF-2 mRNA levels. However this effect was not translated at the protein level. We hypothesize that ICC contains other polyphenols in addition to caffeic acid that are able to increase ATF-2 protein levels through an increase of the translation of its mRNA, the increase of stability of the protein or an inhibition of its degradation. In this direction several plant polyphenols such as (-)-epigallocatechins-3-gallate (EGCG), genistein, luteolin, apigenin, chrysin, quercetin, curcumin, and tannic acid have been described to possess proteasome-inhibitory activity [65, 66].

The regulation of ATF-2 transcriptional activity, mostly at the level of its phosphorylation status, has been described upon treatment of cancer cells with several natural compounds. In MCF-7 cells, the anticancer agent 3,30-Diindolylmethane, derived from Brassica vegetables, activates both JNK and p38 pathways, resulting in c-Jun and ATF-2 phosphorylation, and the increase of binding of the

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**Table 6: Common differentially expressed genes in HT29 treated-cells.**

| Gene symbol | FC absolute ICC | P value | Regulation | FC absolute CA | P value | Regulation |
|-------------|----------------|---------|------------|----------------|---------|------------|
| FST         | 1.343          | 0.025   | Up         | 1.375          | 0.022   | Up         |
| SULT1B1     | 1.349          | 0.018   | Up         | 1.304          | 0.020   | Up         |
| EPOR        | 1.372          | 0.008   | Up         | 1.321          | 0.021   | Up         |
| HINT3       | 2.410          | 0.040   | Up         | 2.607          | 0.033   | Up         |
| STAT5B      | 1.312          | 0.007   | Up         | 1.334          | 0.012   | Up         |
| GLS         | 1.335          | 0.001   | Down       | 1.370          | 0.001   | Down       |
| PPP3R1      | 1.397          | 0.023   | Down       | 1.423          | 0.026   | Down       |
| ATF2        | 1.481          | 0.005   | Down       | 1.354          | 0.016   | Down       |
| SLC4A7      | 1.314          | 0.029   | Down       | 1.322          | 0.025   | Down       |
| MARCH3      | 1.330          | 0.016   | Down       | 1.319          | 0.005   | Down       |
| TBL1XR1     | 1.426          | 0.001   | Down       | 1.332          | 0.001   | Down       |
| SAP30L      | 1.375          | 0.013   | Down       | 1.405          | 0.021   | Down       |
| FGD4        | 1.593          | 0.001   | Down       | 1.523          | 0.027   | Down       |
| ZDHHC20     | 1.665          | 0.004   | Down       | 1.314          | 0.005   | Down       |
| ZDHHC21     | 1.642          | 0.037   | Down       | 1.318          | 0.016   | Down       |
| FAM76B      | 1.506          | 0.014   | Down       | 1.325          | 0.037   | Down       |
| CLK4        | 1.326          | 0.049   | Down       | 1.339          | 0.021   | Down       |

Common differentially expressed genes in HT29 treated-cells with a P value < 0.05 and a minimum fold of 1.3. Column ICC correspond to cells treated with instant caffinated coffee and column CA corresponds to cells treated with caffeic acid. Overexpressed genes are indicated on the upper part of the table, whereas underexpressed genes are depicted in the lower part. The genes in bold, STAT5B and ATF-2, were chosen for further analysis.
Cyclin D1 overexpression has been reported between 40 overexpressing breast cancers are ER positive [24, 25, 81]. It has been shown that the large majority of cyclin D1 – the observation that although cyclin D1 overexpression is rationale for the choice of MCF-7 cell line was based on [71, 72]. Thus CA could represent potential therapeutical cascades involved in intestinal inflammation [69, 70], and pathway is one of the major intracellular signal transduction by blocking the phosphorylation of p38 MAPK and ATF-2 in a dose-dependent fashion [68]. The JNK stress-activated appear to be a good approach for cancer treatment. In this direction our observation that coffee and caffeic acid are able to drastically reduce the expression of cyclin D1 in breast cancer cells could suggest that some coffee components could be used as a coadjuvant therapeutic tool in the treatment of breast cancer.

Abbreviations

APRT: Adenine phosphoribosyltransferase
ATF-2: Activating transcription factor
BAN: Biological association network
CA: Caffeic acid
DMSO: Dimethyl sulfoxide
DEPC: Diethyl pyrocarbonate
ICC: Instant caffeinated coffee
RT-PCR: Reverse transcription-polymerase chain reaction
STAT5B: Signal transducer and activator of transcription 5B.

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