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Research Article

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

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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-caffeoylquinic 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% CO_2 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 H_2O 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 Clínic, Barcelona).

2.4. Microarray Data Analyses. Quantification was carried out with GeneSpring GX v.11.5.1 software (Agilent Technologies), which allows multifilter 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 3 μL 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 manufacturers recommendations. Fold changes in gene expression were calculated using the standard $\Delta\Delta$Ct method.

2.8. Western Blot. Whole extracts were obtained from 2.5×10^6 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 N_2 and stored at -80°C. Total extracts (50 μg) were resolved on SDS-polyacrylamide gels and transferred to PVDF membranes (Immobilon P, Millipore) using a semidry 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 3 (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

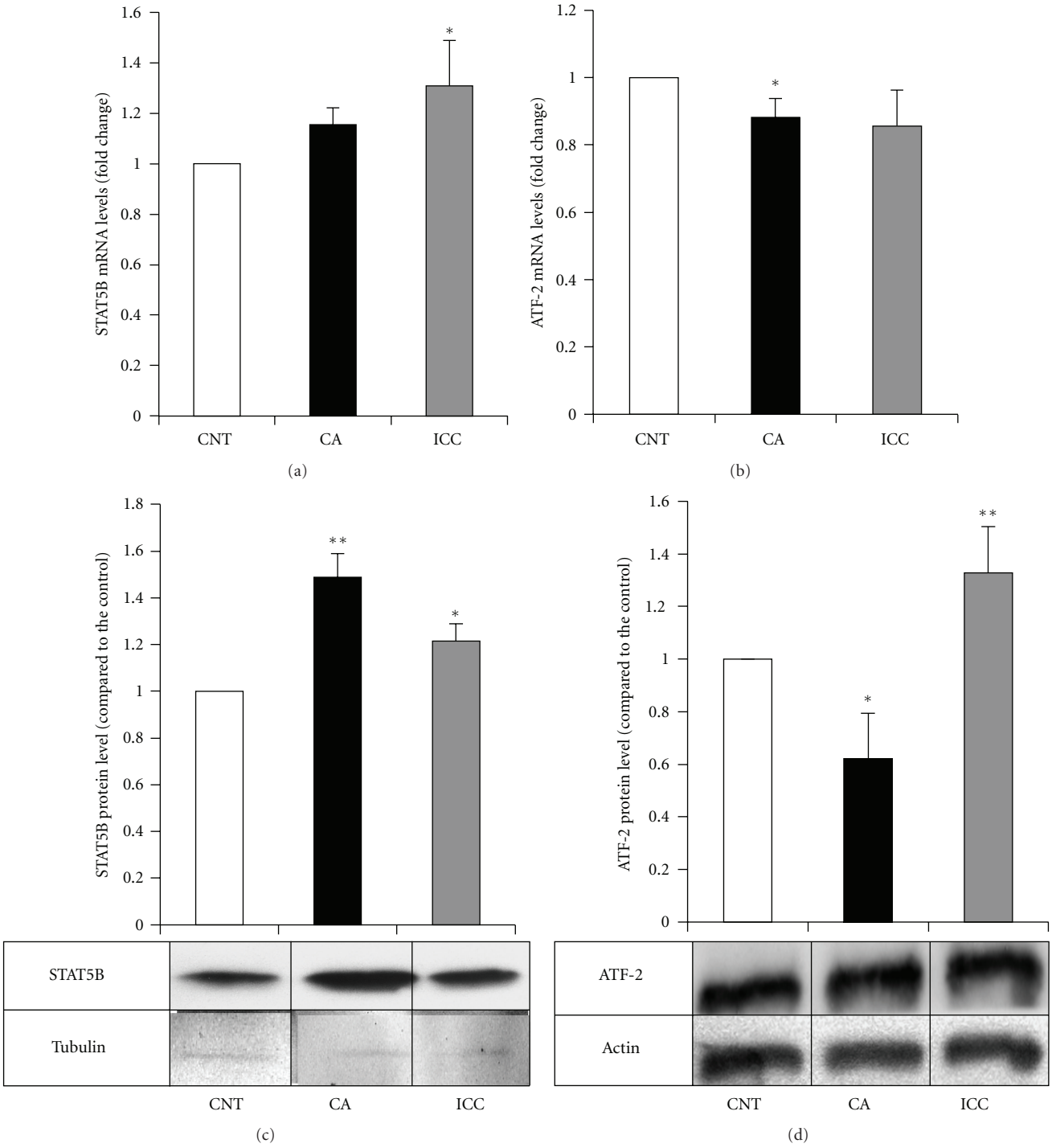

Figure 2: Quantitation of mRNA and protein levels for STAT5B and ATF-2 in HT29 cells. The mRNA levels of STAT5B (a) and ATF-2 (b) were determined in control HT29 cells (empty bars) and cells treated with caffeic acid (CA, filled bars) and instant caffeinated coffee (ICC, grey bars) by RT real-time PCR 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
FGFRL1	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
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	Sulfotransferase 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
ARHGDIA	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).

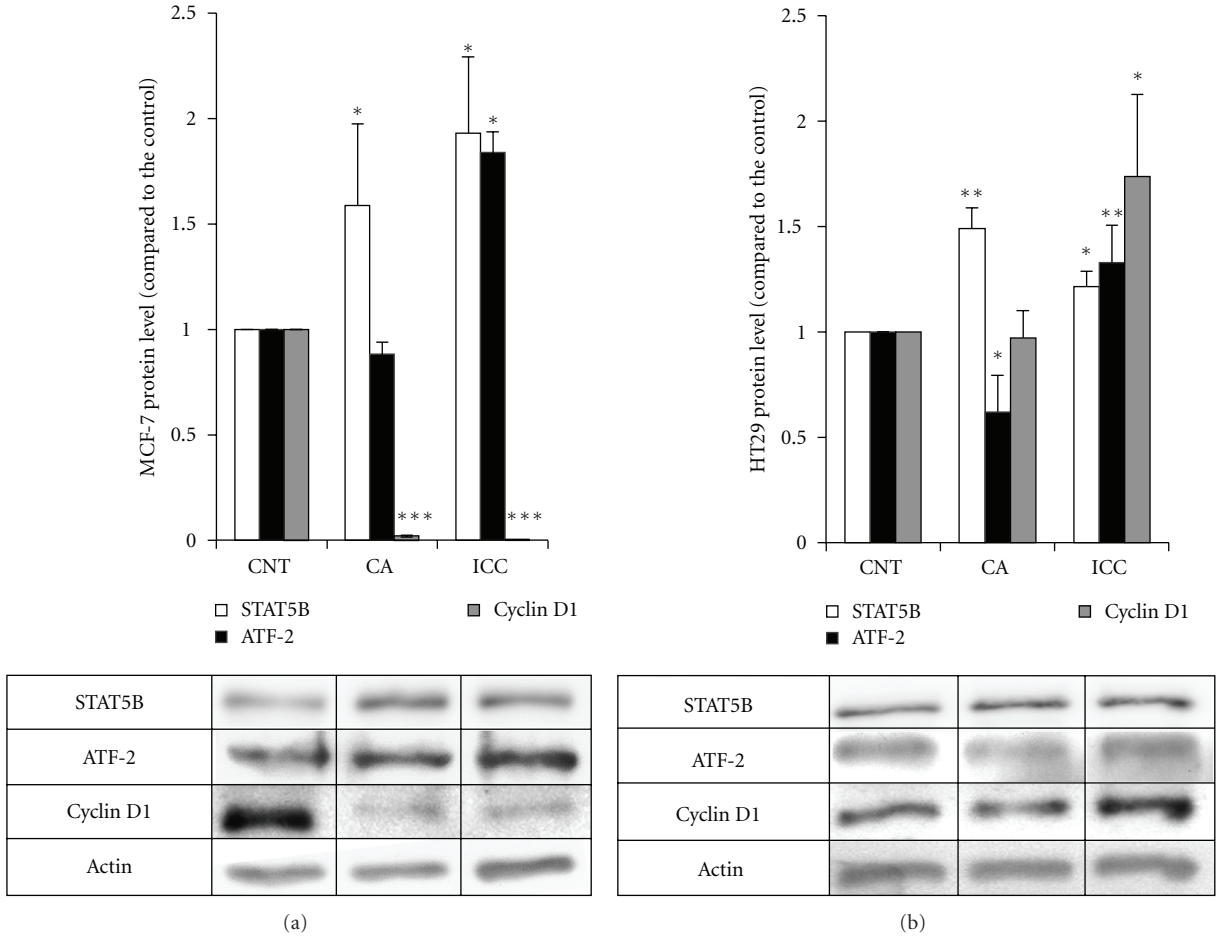

Figure 3: Expression of cyclin D1 upon incubation with ICC and CA in HT29 and MCF-7 cells. (a) Quantitation of STAT5b (empty bars), ATF-2 (filled bars), and cyclin D1 (grey bars) protein levels in MCF-7 cells. The protein levels were determined in control MCF-7 cells (CNT) and cells treated with caffeic 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 $^{***}P < 0.001$ 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	Coxsackie 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 guanylyltransferase 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	1.31	Down
CBL	Cas-Br-M ecotropic retroviral transforming sequence	0.008	1.31	Down
CDC42SE2	CDC42 small effector 2	0.022	1.31	Down
CLN5	Ceroid-lipofuscinosis, neuronal 5	0.001	1.31	Down
DDX3X	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, X-linked	0.027	1.31	Down
FGFR1OP2	FGFR1 oncogene partner 2	0.049	1.31	Down
LRRFIP1	Leucine-rich repeat (in FLII) interacting protein 1	0.026	1.31	Down
PDCD4	Programmed cell death 4	0.005	1.31	Down
REPS2	RALBP1-associated Eps domain containing 2	0.046	1.31	Down
SLC7A6	Solute carrier family 7, member 6	0.002	1.31	Down
TFRC	Transferrin receptor (p90, CD71)	0.038	1.31	Down
TMEM19	Transmembrane protein 19	0.024	1.31	Down
AGPS	Alkylglycerone phosphate synthase	0.001	1.31	Down
SLC4A7	Solute carrier family 4, member 7	0.028	1.31	Down
SPTAN1	Spectrin, alpha, nonerythrocytic 1 (alpha-fodrin)	0.02	1.31	Down
GPD2	Glycerol-3-phosphate dehydrogenase 2 (mitochondrial)	0.033	1.31	Down
BICD1	Bicaudal D homolog 1 (*Drosophila*)	0.008	1.31	Down
FBXW11	F-box and WD repeat domain containing 11	0.025	1.31	Down
BCLAF1	BCL2-associated transcription factor 1	0.025	1.32	Down
CDH1	Cadherin 1, type 1, E-cadherin (epithelial)	0.011	1.32	Down
CLK4	CDC-like kinase 4	0.049	1.32	Down
PTAR1	Protein prenyltransferase alpha subunit repeat containing 1	0.027	1.32	Down
SMEK2	SMEK homolog 2, suppressor of mek1 (*Dictyostelium*)	0.012	1.32	Down
CEPT1	Choline/ethanolamine phosphotransferase 1	0.038	1.32	Down
SAR1A	SAR1 gene homolog A (*S. cerevisiae*)	0.033	1.32	Down
PDGFC	Platelet-derived growth factor C	0.02	1.32	Down
NFAT5	Nuclear factor of activated T-cells 5, tonicity responsive	0.045	1.32	Down
FRS2	Fibroblast growth factor receptor substrate 2	0.03	1.32	Down
BMS1P5	BMS1 pseudogene 5	0.036	1.33	Down
GLS	Glutaminase	5.00E-04	1.33	Down
LMAN1	Lectin, mannose binding, 1	7.00E-04	1.33	Down
ARHGAP18	Rho GTPase-activating protein 18	8.00E-04	1.33	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 13	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-B	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	DCN1, 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 T	0.002	1.35	Down
PEX13	Peroxisome biogenesis factor 13	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
CREBZF	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 (U5 snRNP specific)	0.001	1.36	Down
GLT25D1	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 126	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 (myomegalin)	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

Table 3: Continued.

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
CXADRP1	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-acetylneuraminate 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 metallopeptidase domain 10	0.011	1.43	Down
ADAM9	ADAM metallopeptidase 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 I	0.005	1.44	Down
ZFX	Zinc finger protein, X-linked	0.033	1.44	Down
SGPL1	Sphingosine-1-phosphate lyase 1	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 acetylesterase	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 cDNAs, 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

Table 5: List of underexpressed genes in HT29 cells upon incubation with caffeic acid.

Gene symbol	Gene title	*P* value	FC absolute	Regulation
MFSD7	Major facilitator superfamily domain containing 7	1.00E-04	1.3	Down
MSI2	Musashi homolog 2 (*Drosophila*)	0.027	1.3	Down
CDA	Cytidine deaminase	2.00E-04	1.31	Down
DEFB1	Defensin, beta 1	0.026	1.31	Down
PIP5K1A	Phosphatidylinositol-4-phosphate 5-kinase, type I, alpha	0.027	1.31	Down
ZDHHC20	Zinc finger, DHHC-type containing 20	0.005	1.31	Down
ZDHHC21	Zinc finger, DHHC-type containing 21	0.016	1.31	Down
SLC4A7	Solute carrier family 4, member 7	0.0249	1.32	Down
CEACAM1	Carcinoembryonic antigen-related cell adhesion molecule 1	0.0459	1.32	Down
PDZRN3	PDZ domain containing RING finger 3	0.002	1.32	Down
WDR62	WD repeat domain 62	0.005	1.32	Down
FAM76B	Family with sequence similarity 76, member B	0.036	1.32	Down
TCF21	Transcription factor 21	0.029	1.33	Down
TBL1XR1	Transducin (beta)-like 1 X-linked receptor 1	6.00E-04	1.33	Down
CLK4	CDC-like kinase 4	0.021	1.33	Down
CYP2A13	Cytochrome P450, family 2, subfamily A, polypeptide 13	0.009	1.34	Down
CXCR4	Chemokine (C-X-C motif) receptor 4	0.0488	1.34	Down
ATF2	Activating transcription factor 2	0.0158	1.35	Down
PDE10A	Phosphodiesterase 10A	0.03	1.35	Down
METT10D	Methyltransferase 10 domain containing	0.003	1.35	Down
PRMT2	Protein arginine methyltransferase 2	7.00E-04	1.36	Down
GLS	Glutaminase	5.70E-04	1.37	Down
SLC38A5	Solute carrier family 38, member 5	0.043	1.37	Down
TINAG	Tubulointerstitial nephritis antigen	0.043	1.38	Down
AQP1	Aquaporin 1 (Colton blood group)	0.0221	1.4	Down
JMJD6	Jumonji domain containing 6	0.004	1.4	Down
SAP30L	SAP30-like	0.021	1.4	Down
FGD4	FYVE, RhoGEF, and PH domain containing 4	0.026	1.52	Down
S100A2	S100 calcium-binding protein A2	0.005	1.53	Down
CTSZ	Cathepsin Z	0.045	1.53	Down
SLC4A4	Solute carrier family 4, member 4	9.00E-04	1.54	Down
AGR3	Anterior gradient homolog 3 (*Xenopus laevis*)	0.011	1.69	Down

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

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 caffeinated 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.

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 response to coffee polyphenols in a breast cancer cell line. The MCF-7 cell line was chosen because expression of the prolactin receptor is more often found in estrogen receptor-positive breast tumors [58]. In our conditions, incubation with CA and ICC led to an increase in STAT5B protein levels in MCF-7 cells, and this result could be the basis for a possible inclusion of coffee polyphenols in the diet of breast cancer patients.

ATF-2 is a member of the ATF-cAMP response element-binding protein (CREB) family of transcription factors that can bind to the cAMP response element (CRE) found in many mammalian gene promoters [59, 60]. ATF-2 exhibits both oncogenic and tumor suppressor functions [61]. CREs are found in several genes involved in the control of the cell cycle, for example, the cyclin D1 gene, and ATF-2 binding to this sequence stimulates the transcription of cyclin D1 [30, 31]. ATF-2 mediated cyclin D1 promoter induction can be stimulated by a number of growth-promoting agents, such as estrogen [31], hepatocyte growth factor [62], and regenerating gene product [63]. ATF-2 has been correlated with proliferation, invasion, migration, and resistance to DNA-damaging agents in breast cancer cell lines.

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 caffeine 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

c-Jun–ATF-2 homodimers and heterodimers to the proximal regulatory element of IFN-γ promoter [67]. Biochanin-A, an isoflavone, existing in red clover, cabbage and alfalfa, has an inhibitory and apoptogenic effect on certain cancer cells by blocking the phosphorylation of p38 MAPK and ATF-2 in a dose-dependent fashion [68]. The JNK stress-activated pathway is one of the major intracellular signal transduction cascades involved in intestinal inflammation [69, 70], and upregulation of ATF-2 has been shown in Crohn's disease [71, 72]. Thus CA could represent potential therapeutical properties in different states of intestinal inflammation due to its combined effects on STAT5B and ATF-2 in HT29 cells.

Finally, the modulation of cyclin D1, a target of STAT5B and ATF-2 transcription factors, upon incubation with coffee polyphenols was established in colon and breast cancer cells. Cyclin D1 overexpression is common in colorectal cancer, but the findings regarding its prognostic value are conflicting. In a recent study, positive expression of cyclin D1 protein was detected in 95 of 169 colonic adenocarcinoma specimens, and increased cyclin D1 levels were associated with poorer prognosis [73]. Furthermore, there was a significant correlation between the positive expression of p-Stat5 and cyclin D1 in patients with colonic adenocarcinoma. However, in a second study, cyclin D1 overexpression was associated with improved outcome in a total of 386 patients who underwent surgical resection for colon cancer, classified as TNM stage II or III. Belt et al. [74] showed that low p21, high p53, low cyclin D1, and high AURKA were associated with disease recurrence in stage II and III colon cancer patients. In this context the effect of ICC on cyclin D1 levels could represent either a positive or a negative effect in colon cancer cells, depending on tumor progression. The increase in cyclin D1 levels could represent a marker of better outcome since it has been recently established that cyclin D1 expression is strongly associated with prolonged survival in male colorectal cancer and that lack of cyclin D1 is associated with a more aggressive phenotype in male patients [75]. However, several natural compounds such as anthocyanins, anthocyanidins, apigenin, luteolin, and fisetin have all been described to induce experimentally cell-cycle arrest and apoptosis through the decrease of cyclin D1 levels in HT29 cells [76–80]. In accordance to these data, the increase observed in cyclin D1 levels in HT29 cells upon incubation with ICC could probably be the consequence of the presence of different compounds other than polyphenols in ICC.

In MCF-7 breast cancer cells, cyclin D1 was down-regulated upon incubation with coffee polyphenols. The rationale for the choice of MCF-7 cell line was based on the observation that although cyclin D1 overexpression is present across multiple histologic subtypes of breast cancer, it has been shown that the large majority of cyclin D1–overexpressing breast cancers are ER positive [24, 25, 81]. Cyclin D1 overexpression has been reported between 40 and 90% of cases of invasive breast cancer, while gene amplification is seen in about 5–20% of tumors [24, 81–83]. In cyclin D1-driven cancers, blocking cyclin D1 expression by targeting the cyclin D1 gene, RNA, or protein should increase the chances for therapeutic success. Cell culture studies have raised the possibility that certain compounds might act in this way [84, 85] and approaches to blocking cyclin D1 expression using antisense, siRNA, or related molecules specifically target the driving molecular lesion itself [86–88]. It is believed that compounds that modulate cyclin D1 expression could have a role in the prevention and treatment of human neoplasias. For instance, flavopiridol, a synthetic flavonoid based on an extract from an Indian plant for the potential treatment of cancer, induces a rapid decline in cyclin D1 steady-state protein levels [89]. Taking all these results together, inhibition of cyclin D1 expression appears 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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