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## Data in Brief

## Expression profiling of genes modulated by estrogen, EGCG or both in MCF-7 breast cancer cells

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## ABSTRACT

(–)-Epigallocatechin-3-gallate (EGCG) is one of the most potent and the most studied green tea catechin. Reports on mechanisms of EGCG action and its cellular targets are plenty. Compelling evidences in the literature in favor of ER being one of its targets suggest that EGCG may have a significant impact on estrogen regulated gene expression. Despite the possible implications on breast cancer chemoprevention or therapy, this aspect of EGCG action has not been adequately investigated. In order to address this issue, we have obtained gene expression profiles of MCF-7 breast cancer cells treated with ethanol (vehicle control) and those treated with estrogen, EGCG or both, using microarrays. Here, we have presented in detail the design and execution of the microarray experiment, quality control checks and analysis of microarray data. The utility and importance of the data generated in this work have been discussed in the context of the background literature. Our data is available in the Gene Expression Omnibus (GEO) database with the identifier GSE56245.

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## Specifications

Organism/cell line/tissue	<i>Homo sapiens</i> /ER-positive MCF-7 breast cancer cell line
Sex	Female
Sequencer or array type	Agilent's human gene expression study 8 × 60 K microarray slides (AMADID:27114)
Data format	Raw and processed
Experimental factors	MCF-7 cells treated with 17β-estradiol, EGCG or both.
Experimental features	Total RNA samples isolated from MCF-7 cells treated with 17β-estradiol (10 nM), EGCG (40 μM) or both for a period of 24 h were subjected to microarray analysis using the Agilent platform. Total RNA from cells treated with ethanol (vehicle) served as controls. Eight hybridizations (one color) with eight total RNA samples were carried out. These comprised of total RNA samples from two experimental replicates each of 17β-estradiol, EGCG, 17β-estradiol + EGCG, and ethanol treated cells.
Consent	N/A
Sample source location	Guwahati, Assam, India

## 1. Direct link to deposited data

<http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE56245>.

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## 2. Experimental design, materials and methods

## 2.1. Cell culture

The ER-positive human breast cancer cell line, MCF-7, was routinely cultured in 25 cm<sup>2</sup> flasks (Greiner Bio-One, GmbH, Germany), under standard culture conditions (37 °C, 5% CO<sub>2</sub>), in phenol red containing DMEM-F12 supplemented with 10% heat-inactivated fetal bovine serum (FBS), 100 units/ml of penicillin and 100 μg/ml of streptomycin (M1). Once confluent, the cells were trypsinized and split into fresh 25 cm<sup>2</sup> flasks in a ratio of 1:3 for expansion. Else, the cells were seeded in 35 mm dishes (2 × 10<sup>5</sup> cells per dish) using M1 for experimentation.

## 2.2. Experimental protocol

Once the 35 mm dishes were 60–70% confluent, M1 was replaced with phenol red-free DMEM-F12 supplemented with 10% charcoal stripped heat-inactivated FBS, 100 units/ml of penicillin and 100 μg/ml of streptomycin (M2), and allowed to grow for 4 h. Spent M2 was then replaced with fresh M2 containing ethanol (vehicle), 10 nM of 17β-estradiol (E2), 40 μM of EGCG, or both and incubated further for a period of 24 h before harvesting the cells for total RNA extraction. Thus, the experiment comprised of four treatment groups including the vehicle control. Two dishes (biological replicates for the microarray

analysis) were assigned for each of the treatment groups. The concentrations of E2 and EGCG were optimized earlier [1]. The effectiveness of 10 nM of E2 was confirmed based on the induction of steady state mRNA levels of two estrogen induced genes, namely trefoil factor-1 (pS2) and progesterone receptor (PR). EGCG at 40  $\mu$ M concentration was ideally suited for this experiment, since it caused only a modest (20%) reduction in viability of MCF-7 cells. Under this condition the modulation in gene expression by EGCG could safely be interpreted as primary, and not as a collateral effect of cytotoxicity observed at high concentrations [1].

At the end of the experiment, the cells were lysed in RLT buffer (RNeasy kit, Qiagen, GmbH, Germany). Lysates were sent to Genotypic Technology (P) Ltd., Bangalore, India for total RNA extraction, labeling, hybridization, image acquisition, quality control and primary analysis of raw intensity data.

### 2.3. RNA isolation, labeling, hybridization and image acquisition

RNeasy Mini Kit (Qiagen, GmbH, Germany) was used to extract total RNA. RNA concentrations were determined based on absorbance at 260 nm wavelength of light. The quality of RNA was assessed on 2100 Bioanalyzer (Agilent, Paulo Alto, CA, USA). RNA samples were considered to be of good quality if they satisfied the following criteria – a) A260/A280 ratio > 1.8, b) A260/A230 ratio  $\geq$  1.5, and c) 28S/18S intensity ratio > 1.5. All our total RNA samples satisfied these criteria. The RNA quality control data are provided in Table 1.

For each sample, 500 ng of total RNA was labeled (one color with Cy3) using Agilent's Low Input RNA linear amplification kit (Cat No. 5188–5339). The labeled complementary RNAs were purified using RNeasy Mini Kit, checked for quality (Table 2) and hybridized to Agilent's human gene expression study 8  $\times$  60 K microarray slides (AMADID:27,114), using the Agilent's in situ hybridization kit (Cat No. 5184–3568). Following hybridization and washes, the images were scanned in microarray scanner (Model G2565BA, Agilent). The raw intensity data was extracted using Agilent's Feature Extraction Software.

### 2.4. Assessment of image quality

The images were manually checked for uneven hybridizations, streaks, blobs and other artifacts. Images were found to be clean with low background noise. Microarray images and signal statistics for each array are provided as supplementary information in Appendices A and B, respectively.

### 2.5. Microarray data analysis

For data analysis we used LIMMA package from Bioconductor [2]. Background correction was performed using “normexp” method in LIMMA with an offset of 16 [3]. The “quantile” method was used for normalization of the data between arrays. Further, the “aveeps” function was used to average replicate spots. We applied “lmfit” (linear model) and “eBayes” (Empirical Bayes method) for determination of

**Table 2**  
Quality control of Cy3 incorporation in the labeled RNA.

Sample codes <sup>a</sup>	Cy3 (pmol/ $\mu$ l)	Concentration of labeled RNA (ng/ $\mu$ l)	Absorbance 260/280	Specific activity pmol dye/ $\mu$ g cRNA
C1	1.67	167.56	2.26	9.97
C2	1.02	115.87	2.34	8.80
E1	1.80	176.40	2.28	10.20
E2	1.83	165.75	2.28	11.04
X1	2.25	196.64	2.29	11.44
X2	2.16	202.70	2.31	10.66
EX1	2.01	193.02	2.35	10.41
EX2	1.08	126.66	2.34	8.53

<sup>a</sup> C1 & C2—vehicle treated, E1 & E2—10 nM of 17 $\beta$ -estradiol, X1 & X2—40  $\mu$ M of EGCG, EX1 & EX2—combined treatment with the 10 nM of 17 $\beta$ -estradiol and 40  $\mu$ M of EGCG.

differentially regulated genes. The R code used for processing of the data is as follows:

```
Library (limma)
Targets = readTargets("targets.txt")
x = read.maimages(targets, source = "agilent", green.only = TRUE)
y = backgroundCorrect(x, method = "normexp", offset = 16)
y = normalizeBetweenArrays(y, method = "quantile")
y.ave = aveeps(y, ID = y$genes$ProbeName)
f = factor(targets$Condition, levels = unique(targets$Condition))
design = model.matrix(~0 + f)
colnames(design) = levels(f)
fit = lmFit(y.ave, design)
contrast.matrix = makeContrasts("E-C", "X-C", "EX-C", levels = design)
fit2 = contrasts.fit(fit, contrast.matrix)
fit2 = eBayes(fit2)
output = toptable(fit2, adjust = "BH", coef = "E-C", genelist = y.ave$gene, number = Inf)
write.table(output, file = "E-C.txt", sep = "\t", quote = FALSE)
output1 = toptable(fit2, adjust = "BH", coef = "X-C", genelist = y.ave$gene, number = Inf)
write.table(output1, file = "X-C.txt", sep = "\t", quote = FALSE)
output2 = toptable(fit2, adjust = "BH", coef = "EX-C", genelist = y.ave$gene, number = Inf)
write.table(output2, file = "EX-C.txt", sep = "\t", quote = FALSE).
```

## 3. Discussion

An inverse correlation between green tea consumption and breast cancer risk is apparent from the results of epidemiological studies [4–6]. The widespread interest in the major green tea polyphenol, EGCG, as a chemopreventive and chemotherapeutic agent against breast cancer stems from a substantial amount of data available through experimental studies using cell culture and animal models of breast carcinogenesis [7–10]. Molecular targets of EGCG have also been identified [11]. However, despite the fact that majority of breast tumors at diagnosis are estrogen dependent and ER positive, the impact of EGCG on

**Table 1**  
Total RNA quality control.

Sample codes <sup>a</sup>	Absorbance value 260/280	Absorbance value 260/230	RNA concentration (ng/ $\mu$ l)	Total yield (ng)	QC purity	QC conc./yield	QC integrity
C1	2.1	2.2	324.3	6485.6	Optimal	Optimal	Good
C2	2.1	1.5	260.1	5202.6	Optimal	Optimal	Good
E1	2.1	2.2	336.3	6726.0	Optimal	Optimal	Good
E2	2.1	2.2	337.4	6747.6	Optimal	Optimal	Good
X1	2.1	2.2	359.1	7182.6	Optimal	Optimal	Good
X2	2.1	2.2	410.2	8204.2	Optimal	Optimal	Optimal
EX1	2.1	1.9	283.6	5671.0	Optimal	Optimal	Good
EX2	2.1	2.2	338.7	6773.6	Optimal	Optimal	Good

<sup>a</sup> C1 & C2—vehicle treated, E1 & E2—10 nM of 17 $\beta$ -estradiol, X1 & X2—40  $\mu$ M of EGCG, EX1 & EX2—combined treatment with the 10 nM of 17 $\beta$ -estradiol and 40  $\mu$ M of EGCG.

**Table 3**

A summary of number of probes modulated by E2, EGCG and both.

Treatment <sup>a</sup>	Total number of probes showing modulation <sup>b</sup>	Number of probes showing induction	Number of probes showing repression
E2	3411	1689	1722
EGCG	3317	1844	1473
E2 + EGCG	9183	4998	4185

<sup>a</sup> The concentrations used are 10 nM for E2 and 40 μM for EGCG.<sup>b</sup> The number of probes showing modulation with respect to vehicle treated control.**Fig. 1.** Venn diagram showing the number of probes regulated by the indicated treatments. The concentrations used are 10 nM for E2 and 40 μM for EGCG.

estrogen mediated cellular processes and global gene expression has not been addressed.

Taking a cue from earlier studies which suggest that EGCG can influence estrogen regulated gene expression [12–15], we also demonstrated EGCG mediated increase in mRNA levels of the two estrogen induced transcripts, namely pS2 and PR [1]. The microarray experiment described in this report seeks to address the impact of EGCG on estrogen regulated gene expression at the genome-wide scale. The results of the basic analysis of differentially expressed genes by the treatments with respect to control are presented in Table 3 and Fig. 1. While on one hand there are genes exclusively modulated by each of the treatments, on the other hand a considerable overlap of genes between the treatment groups is also apparent from the data.

This work and the resulting data is a small but significant step towards understanding the mechanism of EGCG action. A comprehensive analysis of the microarray data generated in this study will be useful in obtaining insights into EGCG mediated modulation of estrogen driven processes in ER-positive breast cancer cells. This in turn will help in judging the true potential of EGCG as a chemopreventive or chemotherapeutic agent against breast cancer.

### Conflict of interest

The authors declare no conflict of interest.

### Acknowledgements

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### Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.gdata.2015.05.040>.

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