ELSEVIER

Cancer Letters 182 (2002) 43–51

CANCER Letters

www.elsevier.com/locate/canlet

Gene expression profile in human prostate LNCaP cancer cells by (−) epigallocatechin-3-gallate

Steven I. Wang, Hasan Mukhtar*

Dermatology Department, Case Western Reserve University, 11100 Euclid Avenue, Cleveland, OH 44106-5028, USA

Received 30 November 2001; received in revised form 24 January 2002; accepted 28 January 2002

Abstract

Green tea is an effective chemopreventive agent in animal tumor bioassays and some human cancers. Much of its cancer preventive effects appear to be mediated by its major polyphenolic constituent (−) epigallocatechin-3-gallate (EGCG). In order to better understand the molecular regulation underlying the anti-proliferative activity of EGCG in prostate cancer, we have utilized cDNA microarray to elucidate how EGCG alters program of gene expression in prostate carcinoma LNCaP cells. Fluorophore-labeled cDNA probes synthesized from the untreated LNCaP cells or the cells treated for 12 h with EGCG (12 μM), a physiologically achievable dose, were competitively hybridized to the microarray that contained a total of 250 kinases and phosphatases genes. Such high-throughput screening has identified a number of EGCG-responsive gene candidates. Of these, we found that EGCG induced a subset of genes that functionally could exhibit inhibitory effects on cell growth. The genes repressed by EGCG mostly belonged to the G-protein signaling network. Interestingly, the protein kinase C-α (PKC-α) form, whose inhibition of expression has been shown to inhibit cell growth in some cancer cells, was selectively repressed by EGCG while the expression of six other PKC isoforms (β, δ, ϵ, μ, η and ζ) was unaffected. These EGCG-responsive genes may provide key insights from which to understand mechanisms of action of other polyphenolic compounds in prostate cancer chemoprevention.

Keywords: Cancer prevention; Microarray analysis; (−) Epigallocatechin-3-gallate; Green tea

1. Introduction

Green tea is a popular beverage in some parts of the world. In recent years cancer chemopreventive and chemotherapeutic effects of green tea in cell culture systems and in many animal tumor bioassay systems are well described (refs. [1,2] and references therein). The relevance of these data to human cancer is appreciated for the fact that some studies suggest that green tea consumption by the human population is associated with reduced cancer risk at some body sites (refs. [3,4] and references therein). Much of its cancer preventive effects appear to be mediated by the polyphenols, most notably (−) epigallocatechin-3-gallate (EGCG) present therein. Our most recent studies have shown that oral infusion of a polyphenolic mixture of green tea to TRAMP mice, a mouse model that emulates human prostate cancer progression, results in remarkable chemopreventive effects [5].

Our earlier studies using tissue culture models showed that EGCG causes dysregulation of cell cycle and apoptosis in human prostate cancer cells [1,2]. Biological effects of EGCG have also been studied at the cellular and tissue levels by other

* Corresponding author. Tel.: +1-216-368-1127; fax: +1-216-368-0212.

E-mail address: hxm4@po.cwru.edu (H. Mukhtar).

0304-3835/02/$ - see front matter
PII: S0304-3835(02)00065-4

laboratories where they show the ability of EGCG to modulate endocrine systems [6] and activity of some signaling proteins such as STAT1 ([7] and references therein), and regulate expression of a number of genes ([2] and references therein). Nuclear factors such as Sp-1 and NF-κB have been implicated in the involvement of EGCG regulation [8,9].

Regulatory events at the molecular level that govern the cancer preventive effect of EGCG remain to be delineated. Here, for the first time, using cDNA microarray technology, we determined the effect of EGCG on enhancement and repression of transcription of genes that have biological functions in cellular regulatory pathways. Our results suggest that transcriptional regulation of some signaling proteins is also part of the regulatory events by which EGCG initiates its anti-proliferative effect in prostate cancer.

2. Materials and methods

2.1. Tissue culture

Human prostate carcinoma cells LNCaP were maintained in RPMI medium with 10% fetal bovine serum (Hyclone) and streptomycin and penicillin at 37°C in a humidified chamber. Approximately 1×10^6 cells per T75 flask were set up in duplicate and grown for 4–5 days to 80% confluence. For the treated cells, EGCG dissolved in water was added in the medium to a final concentration of 12 μM and cells were incubated for an additional 12 h before harvest. Only water was added to the control flask.

2.2. Probe preparation and hybridization

Total RNA from EGCG-treated or control LNCaP cells was purified using RNeasy Mini kit (Qiagen, Inc.) with a modified procedure. In brief, the cells were directly dissolved in RNAwiz reagent (Ambion, Inc.) and chloroform followed by centrifugation at $10\,000 \times g$ for 15 min. The aqueous phase was carefully transferred to a fresh tube and equilibrated with one volume of 70% ethanol before loading into the Qiagen column to proceed the total RNA purification.

Reagents provided in the Micromax Direct System (MPS201001KT, Perkin Elmer Life Sciences) were used for cDNA synthesis and cyanine labeling. Poly(A) RNA from 120 μg of total RNA of the treated or control cells was converted to cDNA by reverse transcriptase, from which the EGCG-treated cDNA was labeled with Cyanine-3 whereas the control cDNA was incorporated with Cyanine-5. Both Cyanine-3 and -5 probes were combined and co-purified using Microcon 100 membrane cartridges according to manufacturer's procedure.

Prior to hybridization, half of the combined probes were desiccated by speed vacuum and resuspended in 20 μl hybridization buffer provided in the kit. The denatured probes were added to the microarray and incubated at 65°C overnight. The washing procedure included twice with $0.5 \times$ SSC and 0.01% SDS, once with $0.06 \times$ SSC and 0.01% SDS, and finally once with $0.06 \times$ SSC only, each time 30 ml at room temperature for 15 min. The microarray image was scanned and quantified by Perkin Elmer Life Sciences. For this study, the microarray experiments were repeated at least twice, each time of which the hybridizing templates were generated from a fresh batch of cells for treatment.

2.3. Analysis of expression data and selection of genes with different expression

To ensure maximal gene expression to be detected, cDNA microarray was scanned using low/medium settings of laser power and photomultiplier voltage followed by a scan at high laser power. Gene expression level for each channel was quantified by pixels of a circle positioned on every gene spot in the microarray. Outside the circle, pixels between two rings at the predetermined distance from the border of the spot circle were defined as local background. Differences in gene expression were compared after subtraction of local background for every gene spot and for every channel separately. In order to compare the expression of each gene in response to the EGCG treatment, intensity values for all genes in the Cyanine 5 channel were normalized to the Cyanine 3 channel, from which the Cyanine 3/5 expression ratios were calculated that reflected the magnitude of induction of gene expression by EGCG. The reciprocal values were calculated to establish the magnitude of gene repression by EGCG. According to the manufacturer, only ratios at least higher than two indicated a significant change in expression and

such criteria were used to select the EGCG-responsive genes in this study.

2.4. Slot blotting analysis

Reverse transcriptase-polymerase chain reactions (RT-PCR) using cDNA templates synthesized from HeLa cells were carried out to obtain the probes for protein kinase C-α (PKC-α) and tyrosine receptor kinase E (Trk E). The cDNA templates were synthesized as following: poly(A) RNA from approximately 1×10^6 HeLa cells was purified using QuickPrep Micro mRNA purification kit (Amersham). A total of 25 ng of poly(A) RNA was mixed with 4 μg of hexamers and 1 μg of single-stranded binding protein to a total volume of 25 μl and incubated at 70°C for 30 m. The reverse transcription contained 1× first-strand buffer, 10 mM DTT, 2 mM dNTP, 0.2 U RNAse inhibitor and 300 U reverse transcriptase (Gibco Life Technologies) and the reaction was incubated at 42°C for 2 h. The primers used in the PCR reaction [10] for each probe were as following: for PKC-α, 5′-GGACTGGGATCGAACAAC A-3′ and 5′-CATCCATCATGT GTTCCTTG-3′; for Trk E, 5′-CTTGCAGGTGGATCTACAAC-3 and 5′-GGAATTGTTCACCACATCAGA-3′, all of which were derived from GenBank X52479 and X74979, respectively. The PCR product was purified and verified by sequencing. Both probes were labeled using BrightStar psoralen-biotin non-isotopic labeling kit (Ambion Inc.) and purified by Microcon-PCR Filter Unit (Millipore).

For slot blotting, mRNA from EGCG-treated or control LNCaP cells was purified using QuickPrep Micro mRNA purification kit (Amersham). A total of 1 μg of denatured mRNA in 5× TBE was loaded into the slot using the Schleicher & Schuell 48-well Slot-Blot Manifold. The blot was sequentially washed once with 5× TBE then with 0.5× TBE prior to UV cross-linking. The hybridization was carried out in 5 ml UltraHyb solution (Ambion) at 42°C overnight. After thorough washes with low and high stringency solutions (Ambion), the hybridization signals were detected using BrightStar BioDetect non-isotopic detection kit (#1930, Ambion) according to manufacturer's procedure. Each image was obtained from a 2-h exposure.

3. Results and discussion

3.1. A high-throughput approach to reveal gene regulation by EGCG

Molecular regulation underlying the cancer chemoprevention of EGCG in prostate cancer remains unclear. In order to better understand the molecular regulation underlying the anti-proliferative activity of EGCG in prostate cancer, we have utilized cDNA microarray technology to elucidate how EGCG alters program of gene expression in human prostate carcinoma LNCaP cells. We hypothesized that such high-throughput approach should reveal the identity of the regulatory genes involved in the EGCG-mediated anti-proliferative activity.

Our first attempt was to explore a total of 250 kinases and phosphatases whose biological functions are associated with a variety of known signal transduction pathways as well as genes functionally involved in regulations such as cell cycle, apoptosis, metabolic biosynthesis; human EST clones that contain a kinase or phosphatase domain were also included in the microarray (the complete gene list can be found in the web site at www.nen.com/products/clone_FA1.html). LNCaP cells were treated for 12 h with or without 12 μM of EGCG, a physiologically achievable dose. The fluorophore-labeled cDNA probes, i.e. the EGCG-treated cDNA labeled with Cyanine-3 and the control cDNA with Cyanine-5, were mixed and added to the microarray where competitive binding for each gene expressed from two distinct populations to its corresponding gene spot in the microarray was carried out.

A representative image from such cDNA microarray is shown in Fig. 1. A false red color overlay was assigned to each gene spot in the Cyanine-3 channel whereas a false green color overlay was given to the Cyanine-5 channel. The resulting compound color for each spot could dynamically range from red to green where yellow color reflected equal gene expression (Fig. 1). Further analysis revealed a sum of 25 genes that showed a significant response to EGCG (Table 1). Of these, there were sixteen genes whose expression was significantly increased by EGCG, and nine genes whose expression was significantly reduced. Interestingly, these EGCG-responsive gene candidates are functionally diverse and do not share a common regu-

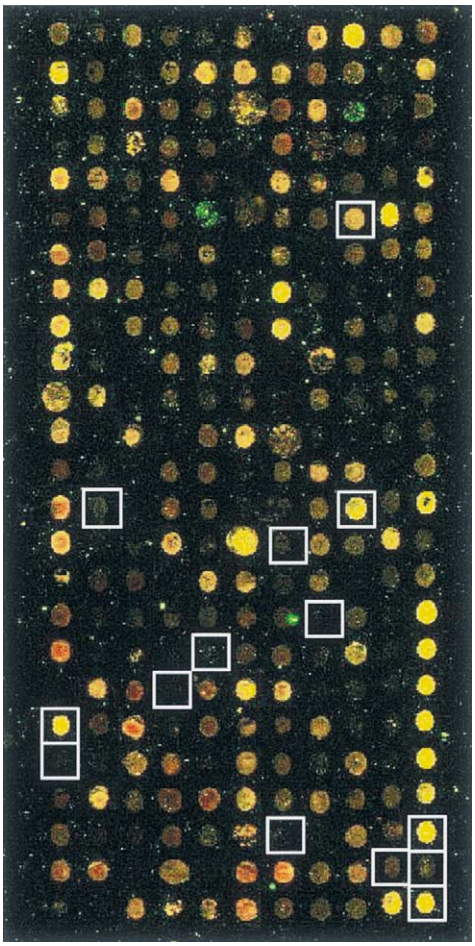

Fig. 1. A representative microarray image composed of a total of 250 kinases and phosphatases. Each gene spot contained a full-length cDNA that was immobilized on the glass slide. Non-mammalian genes as the internal control spots were indicated in square frames. Total RNA was purified and converted to cDNA from human prostate carcinoma LNCaP cells treated with 12 μM EGCG or water-only for 12 h. The EGCG-treated cDNA was labeled with Cyanine-3 to which a false red color overlay was assigned, whereas the control cDNA was incorporated with Cyanine-5 to which a false green color overly was assigned. After scanning for each channel, the resulting compound color (shown) could range from red to green where yellow color indicated equal gene expression of the corresponding gene.

latory pathway, suggesting that molecular regulation underlying the effect of EGCG probably requires a combination of various regulatory components in the cell to achieve the effect. These EGCG-responsive genes may provide key insights from which to understand mechanisms of action of other polyphenolic compounds in prostate cancer chemoprevention.

The majority (90%) of the genes in the microarray were not remarkably affected by EGCG during the

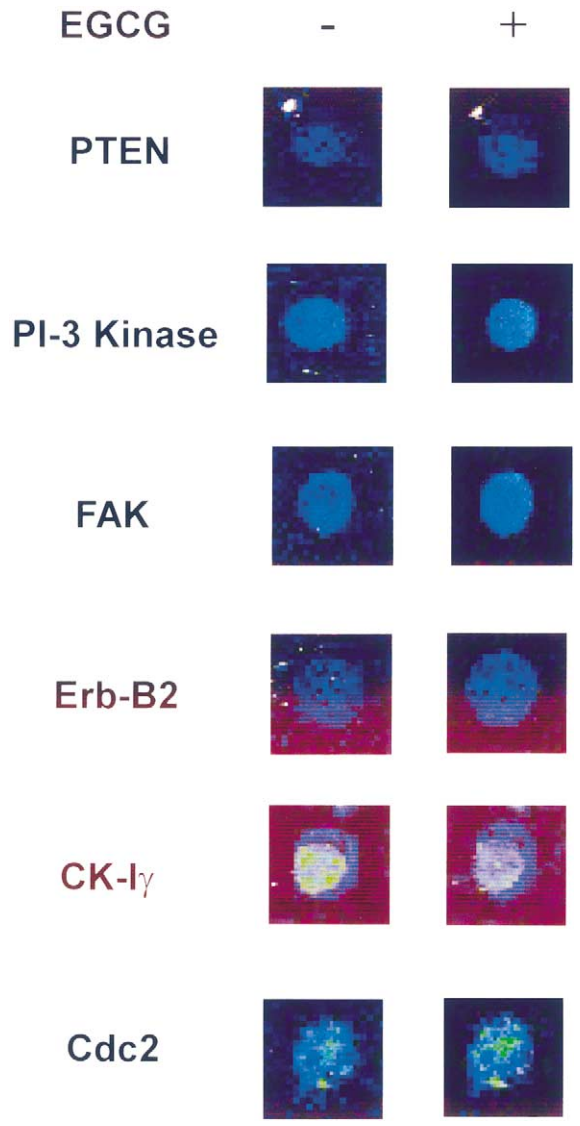

Fig. 2. Examples of kinases and phosphatases whose expression was unaffected by EGCG in LNCaP cells. To facilitate visualization of the gene expression in the Cyanine-3 and -5 channels separately, a false blue color overlay was assigned to each channel after intensity values for all genes in the Cyanine-5 channel were normalized to the Cyanine-3 channel. cdc2, cell-division-cycle 2 gene; PI-3 kinase, phosphatidylinositol-3 kinase; FAK, focal adhesion kinase; CK, casein kinase.

first 12 h treatment in LNCaP cells, indicating that these non-responsive genes are not the transcriptional regulatory targets in the early stage of EGCG-mediated effect. For instance, the expression of two key regulatory proteins in the phosphatidylinositol-3 kinase signaling pathway, PI-3 kinase and the dual-specificity phosphatase PTEN, was not enhanced or reduced during the course of the treatment despite the absence of Akt in the microarray (Fig. 2). EGCG did not have any influence on the expression of lipid kinase family such as phosphoinositide 3-kinase and phosphatidylinositol 4-kinase that are important for phospholipid metabolism and intracellular calcium equilibrium (data not shown).

Table 1
Candidate genes responsive to (−) epigallocatechin-3-gallate in human prostate cancer LNCaP cells[a]

Gene response	Accession #
A. Repression	
Protein kinase C-α[b]	X52479
41 kDa protein kinase related to rat ERK2[b]	Z11694
Type I β cGMP-dependent protein kinase[b]	Y07512
Adenosine kinase short form	U90339
Phosphatidylinositol 3-kinase homolog	U26455
Protein tyrosine phosphatase PIR1	AF023917
Protein tyrosine phosphatase zeta	M93426
KIAA0369 gene	AB002367
Leukocyte common antigen T200	Y00638
B. Induction	
Tyrosine receptor kinase type E mRNA[b]	X74979
Phosphoglycerate kinase	V00572
Adenylate kinase 2A	U84371
CDK8 protein kinase	X85753
Putative serine/threonine protein kinase	Y10032
Ribosomal protein kinase B	AJ10119
Mevalonate kinase	M88468
Protein tyrosine phosphatase	M25393
Prostatic acid phosphatase	M34840
Receptor-type protein tyrosine phosphatase γ	L09247
Protein tyrosine phosphatase 1C	X62055
STE-20 related kinase SPAK	AF099989
IAR/receptor-like protein tyrosine phosphatase	AF007555
Pyrroline 5-carboxylate synthase	X94453
Glomerular epithelial protein 1	U20489
Platelet-derived growth factor A type receptor	M22734

[a] Based on at least two independent hybridization experiments. The complete list of genes in the microarray can be found in the web site at http://www.nen.com/products/clone_FA1.html.
[b] Response detected in both experiments.

Focal adhesion kinase and LIM kinase are important for regulation of cell migration and motility [11,12] and they were not a primary target of EGCG, either. Expression of growth factor receptors such as erbB2/neu and fibroblast growth factor receptor, or nuclear kinases such as the casein kinase family whose biological role is associated with regulation of membrane trafficking and signal transduction [13] did not show significant changes (Fig. 2). Although EGCG could cause dysregulation of cell cycle in prostate cancer cells, it had very little effect on the expression of cdc2 (Fig. 2) and some cyclin-dependent protein kinase (U79269) though more thorough evaluation on this aspect needs to be conducted. Overall, we speculate that post-translational control of these proteins is possibly a more important level of regulation than transcription.

3.2. Repression of gene expression by EGCG

We have identified nine genes, including six kinases and three phosphatases, whose expression was found to be down-regulated by EGCG (Table 1). Inspection of the relationship of these repressed regulatory genes revealed that except for KIAA0369 (AB002367), leukocyte common antigen T200 (Y00638) and protein tyrosine phosphatase PIR1 (AF023917), the remaining six genes were related to the G-protein signaling network. For EGCG to exhibit its anti-proliferative activity in prostate cancer cells, expression of adenosine kinase (U90339), PKC-α (X52479) and type I β cGMP-dependent protein kinase (Y07512) was likely inhibited by EGCG to be able to block the intracellular cyclic-nucleotides signaling cascade. The expression of protein tyrosine phosphatase zeta (M93426) whose activation could activate PI-3 kinase and further MAP kinases that are downstream regulators of the G-protein signal transduction was repressed to antagonize cell migration [14]. The ERK2 homolog (Z11694) and PI-3 kinase homolog (U26455) bearing functional similarity to their respective prototype may have a role in prostate cells in inhibition of cell growth activated by the MAPK and PI-3 kinase pathways.

Of twelve PKC isoforms identified to this date (reviewed in ref. [15]), seven isoforms (α, β, δ, μ, ϵ, δ, and η) were present in the microarray (Fig. 3A). The expression of the PKC-α form was selec-

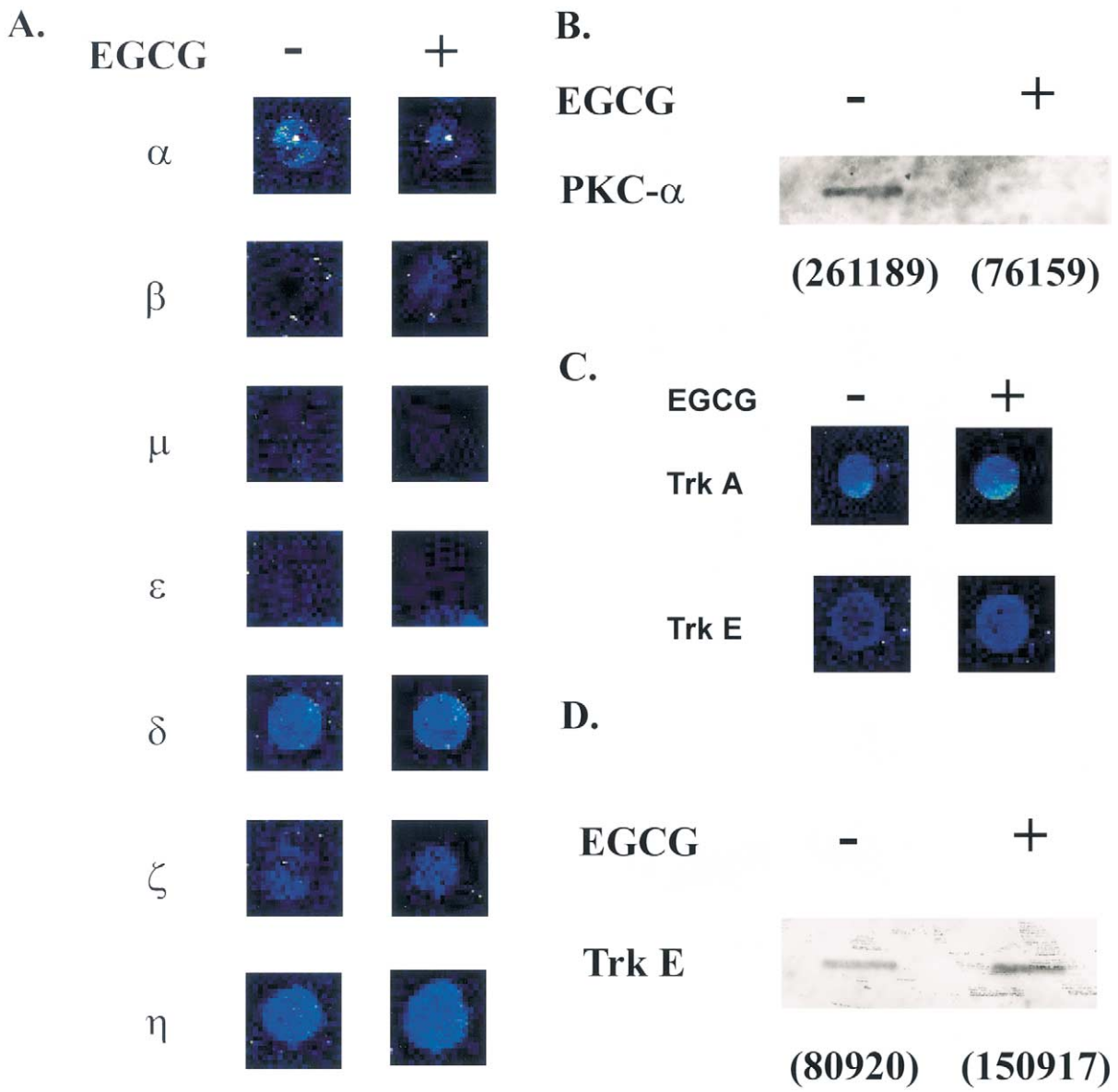

Fig. 3. Verification of the PKC-α and Trk E gene expression in response to EGCG by RNA slot blot hybridization. (A) The cDNA microarray identified the PKC-α form as the transcriptional down-regulation target in the PKC gene family during the EGCG treatment in LNCaP cells. Seven PKC isoforms were present in the microarray and their respective response to the EGCG treatment was shown. (B) Slot blot hybridization confirmed the repression of PKC-α gene expression by EGCG. (C) Two members of the Trk gene family, types A and E, were present in the microarray, and only Trk E was induced by EGCG. (D) Slot blot hybridization confirmed the induction of Trk E gene expression by EGCG. The intensity of each slot was quantified by the UN-SCAN-IT program and the intensity values in pixels were indicated. The experiment was repeated at least once with similar results.

tively repressed by EGCG while the expression of other six isoforms was not affected (Fig. 3A). The PKC-α gene repression by EGCG was also validated by RNA slot blotting hybridized with a PKC-α specific probe, whose sequence was derived from the 762–1501 region of the cDNA that showed very little

homology to other PKC isoforms (Fig. 3B). The result showed that EGCG reduced the PKC-α gene expression by approximately three folds.

The presence of PKC-α in the gene repression list is intriguing because PKCs are involved in diverse cellular functions including cell differentiation, growth control, tumor promotion and cell death (ref. [15] and references therein). PKC is also a regulator of cell cycle events during G1 progression and G2/M transition [15]. Distribution of PKC isoforms is tissue specific and the role of a particular PKC isoform can be different from one cell type to another [15]. Recent studies demonstrate that inhibition of PKC-α gene expression could inhibit cell proliferation in the animal tumor model and in some human cancer cell lines (ref. [15] and references therein). Consistent with this notion, EGCG is now shown to inhibit the PKC-α gene expression in LNCaP cells as part of its molecular mechanism of chemoprevention.

3.3. Induction of gene expression by EGCG

Our cDNA microarray detected 16 kinase and phosphatase gene candidates in prostate cells whose expression was enhanced by EGCG (Table 1). The induction of receptor-type protein tyrosine phosphatase-γ gene expression (L09247), a tumor suppressor gene candidate frequently deleted in some human cancers [16,17], may have a major role in prostate cancer prevention mediated by EGCG. Protein tyrosine phosphatase 1C (X62055), also known as SHP-1 that contains two SH2 domains, is also a tumor suppressor gene candidate and plays a role for terminating growth factor and cytokine signals by dephosphorylating critical molecules [18]. Prostatic acid phosphatase (M34840) might help inhibit growth rate by deactivation of erbB-2 and p38 MAP kinase [19]. Pyrroline-5-carboxylate, an endogenous proline-derived metabolite, has been shown to inhibit cell proliferation and survival in some cancer cells [20], and an increase in the gene expression of pyrroline 5-carboxylate synthase (X94453) by EGCG might display similar inhibitory effects in prostate cancer cells. Maintenance of regular cell volume is important for ensuring proper cellular functions. The inductive response of putative serine/threonine protein kinase (Y10032), which is sensitive to cellular hydration status that affects metabolic control and whose expression becomes inducible upon cell shrinkage [21], suggests that EGCG might also involve cell volume control in prostate cancer cells. Furthermore, EGCG seemingly had a slight impact on metabolic biosynthesis as indicated by the response of mevalonate kinase (M88468), phosphoglycerate kinase (V00572) and ribosomal protein kinase B (AJ010119) which is a nuclear protein in part associated with the regulation of glycogen metabolism [22]. As EGCG appears to down regulate the G-protein signaling network, cell proliferation signal activated by platelet-derived growth factor receptor-α (M22734) might be compromised.

Five genes whose regulatory relationship to the cell growth control is not well understood to this date were found to be induced by EGCG and these included Trk E (X74979), adenylate kinase 2A (U84371), protein tyrosine phosphatase (M25393), IAR/receptor-like protein tyrosine phosphatase (AF007555) and glomerular epithelial protein-1 (U20489). The prototype of Trk E, Trk type A, is a proto-oncogene and functions as a transmembrane receptor to which endogenous nerve growth factor binds and signals the onset of cell proliferation (reviewed in ref. [23]). While Trk E is structurally similar to Trk A, in presence of EGCG, Trk E gene expression was induced but not Trk A (Fig. 3C). The Trk E gene expression was increased by approximately two folds in our slot blotting analysis (Fig. 3D). Since neurotrophin receptors can functionally inhibit each other's action to mediate neurotrophin effects [23], we speculate that EGCG-induced Trk E gene expression may negatively modulate the proliferation signal in prostate cells.

Overall, we show that a combination of various cellular regulatory components is required for EGCG to exert its chemopreventive effect against prostate cancer as 25 EGCG-responsive gene candidates (Table 1) important for the molecular regulation underlying the anti-proliferative effect of EGCG against prostate cancer are revealed. Of these, most of the EGCG-repressed gene candidates are broadly related to the G-protein signaling network, therefore implicating a portion of the G-protein signaling network in the early stage of prostate cancer prevention. EGCG also induces several kinases and phosphatases that functionally could exhibit inhibitory effects on prostate cell growth (Table 1). Beside the effect of EGCG on growth inhibition, the presence of two

EGCG-responsive gene candidates, protein tyrosine phosphatase zeta (M93426) and putative serine/threonine protein kinase (Y10032), implies a potential role for EGCG in regulation of cell migration and cell volume control. Moreover, functional diversity of these responsive gene candidates illustrates the complex nature of molecular interaction during the early stage of prostate cancer prevention by EGCG. These genes may prove useful for therapeutic targets for prostate cancer.

Acknowledgements

We thank Dr Nihal Ahmad for his careful reading on this manuscript. Steven I. Wang is a NRSA post-doctoral fellow supported by USPHS grant NIH T32CA59366. This study used resources of P-30AR39750 and was supported by USPHS grant CA78809.

References

[1] N. Ahmad, D.K. Feyes, A.L. Nieminen, R. Agarwal, H. Mukhtar, Green tea constituent epigallocatechin-3-gallate and induction of apoptosis and cell cycle arrest in human carcinoma cells, J. Natl. Cancer Inst. 89 (1997) 1881–1886.

[2] S. Gupta, N. Ahmad, A.L. Nieminen, H. Mukhtar, Growth inhibition, cell-cycle dysregulation, and induction of apoptosis by green tea constituent (−)-epigallocatechin-3-gallate in androgen- sensitive and androgen-insensitive human prostate carcinoma cells, Toxicol. Appl. Pharmacol. 164 (2000) 82–90.

[3] V.W. Setiawan, Z.F. Zhang, G.P. Yu, Q.Y. Lu, Y.L. Li, M.L. Lu, M.R. Wang, C.H. Guo, S.Z. Yu, R.C. Kurtz, C.C. Hsieh, Protective effect of green tea on the risks of chronic gastritis and stomach cancer, Int. J. Cancer 92 (2001) 600–604.

[4] M. Inoue, K. Tajima, M. Mizutani, H. Iwata, T. Iwase, S. Miura, K. Hirose, N. Hamajima, S. Tominaga, Regular consumption of green tea and the risk of breast cancer recurrence: follow-up study from the Hospital-based Epidemiologic Research Program at Aichi Cancer Center (HERPACC), Jpn. Cancer Lett. 167 (2001) 175–182.

[5] S. Gupta, K. Hastak, N. Ahmad, J.S. Lewin, H. Mukhtar, Inhibition of prostate carcinogenesis in TRAMP mice by oral infusion of green tea polyphenols, Proc. Natl. Acad. Sci. USA 98 (2001) 10350–10355.

[6] Y.H. Kao, R.A. Hiipakka, S. Liao, Modulation of endocrine systems and food intake by green tea epigallocatechin gallate, Endocrinology 141 (2000) 980–987.

[7] M. Menegazzi, E. Tedeschi, D. Dussin, A.C. De Prati, E. Cavalieri, S. Mariotto, H. Suzuki, Anti-interferon gamma action of epigallocatechin-3-gallate mediated by specific inhibition of STAT1 activation, FASEB J. 15 (2001) 1309–1311.

[8] F. Ren, S. Zhang, S.H. Mitchell, R. Butler, C.Y. Young, Tea polyphenols down-regulate the expression of the androgen receptor in LNCaP prostate cancer cells, Oncogene 19 (2000) 1924–1932.

[9] N. Ahmad, S. Gupta, H. Mukhtar, Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappaB in cancer cells versus normal cells, Arch. Biochem. Biophys. 376 (2000) 338–346.

[10] S.I. Wang, J. Puc, J. Li, J.N. Bruce, P. Cairns, D. Sidransky, R. Parsons, Somatic mutations of PTEN in glioblastoma multiforme, Cancer Res. 57 (1997) 4183–4186.

[11] M. Tamura, J. Gu, K. Matsumoto, S. Aota, R. Parsons, K.M. Yamada, Inhibition of cell migration, spreading, and focal adhesions by tumor suppressor PTEN, Science 280 (1998) 1614–1617.

[12] M. Maekawa, T. Ishizaki, S. Boku, N. Watanabe, A. Fujita, A. Iwamatsu, T. Obinata, K. Ohashi, K. Mizuno, S. Narumiya, Signaling from Rho to the actin cytoskeleton through protein kinases ROCK and LIM-kinase, Science 285 (1999) 895–898.

[13] S.D. Gross, R.A. Anderson, Casein kinase I: spatial organization and positioning of a multifunctional protein kinase family, Cell Signal. 10 (1998) 699–711.

[14] M. Qi, S. Ikematsu, N. Maeda, K. Ichihara-Tanaka, S. Sakuma, M. Noda, T. Muramatsu, K. Kadomatsu, Haptotactic migration induced by midkine: involvement of protein-tyrosine phosphatase {zeta}, mitogen-activated protein kinase and phosphatidylinositol 3-kinase, J. Biol. Chem. 276 (2001) 15868–15875.

[15] E. Livneh, D.D. Fishman, Linking protein kinase C to cell-cycle control, Eur. J. Biochem. 248 (1997) 1–9.

[16] I. Panagopoulos, N. Pandis, S. Thelin, C. Petersson, F. Mertens, A. Borg, U. Kristoffersson, F. Mitelman, P. Aman, The FHIT and PTPRG genes are deleted in benign proliferative breast disease associated with familial breast cancer and cytogenetic rearrangements of chromosome band 3p14, Cancer Res. 56 (1996) 4871–4875.

[17] K.K. Wary, Z. Lou, A.M. Buchberg, L.D. Siracusa, T. Druck, S. LaForgia, K. Huebner, A homozygous deletion within the carbonic anhydrase-like domain of the Ptprg gene in murine L-cells, Cancer Res. 53 (1993) 1498–1502.

[18] G.S. Feng, T. Pawson, Phosphotyrosine phosphatases with SH2 domains: regulators of signal transduction, Trends Genet. 10 (1994) 54–58.

[19] X.Q. Zhang, M.S. Lee, S. Zelivianski, M.F. Lin, Characterization of a prostate-specific tyrosine phosphatase by mutagenesis and expression in human prostate cancer cells, J. Biol. Chem. 276 (2001) 2544–2550.

[20] S.A. Maxwell, G.E. Davis, Differential gene expression in p53-mediated apoptosis-resistant versus apoptosis-sensitive tumor cell lines, Proc. Natl. Acad. Sci. USA 97 (2000) 13009–13014.

[21] S. Waldegger, P. Barth, G. Raber, F. Lang, Cloning and characterization of a putative human serine/threonine protein kinase transcriptionally modified during anisotonic and isotonic alterations of cell volume, Proc. Natl. Acad. Sci. USA 94 (1997) 4440–4445.

[22] M. Tomas-Zuber, J.L. Mary, F. Lamour, D. Bur, W. Lesslauer,

C-terminal elements control location, activation threshold, and p38 docking of ribosomal S6 kinase B (RSKB), J. Biol. Chem. 276 (2001) 5892–5899.

[23] D.R. Kaplan, F.D. Miller, Neurotrophin signal transduction in the nervous system, Curr. Opin. Neurobiol. 10 (2000) 381–391.