Available online at www.sciencedirect.com
SCIENCE DIRECT®
Mutation Research 556 (2004) 193–200
Fundamental and Molecular Mechanisms of Mutagenesis
www.elsevier.com/locate/molmut
Community address: www.elsevier.com/locate/mutres

Gene expression spectra in human leukemia HL-60 cells treated with EGCG

J. Cao[a,*], L.L. Ren[a], J.W. Liu[a], Y. Li[a], H.Y. Qu[b]

[a] *Tea and Health Laboratory, Central-South University Xiangya School of Medicine, Changsha 410078, Hunan, PR China*
[b] *Integrate Traditional Chinese and Western Medicine Hospital, Beijing, China*

Received 14 February 2004; received in revised form 23 April 2004; accepted 17 August 2004

Abstract

To decipher the molecular mechanism of EGCG induced HL-60 cell apoptosis, alterations of gene expression spectra in HL-60 cell line cells after treatment with EGCG were screened by cDNA chip, and analyzed with the GenePix 3.0 twice; and the cDNA chip results further identified by RT-PCR. Ninety-seven genes among the total 8398 (1.15%) showed consistent significant differential expression in the duplicated cDNA chip assessments. Thirty-nine genes (40.2%) were up-regulated and 58 genes (59.8%) were down-regulated; and the randomly selected four performed RT-PCR results agreed with the cDNA chip data. The results suggest that the apoptosis of HL-60 cells induced by EGCG is a progressive transformation process regulated by a variety of genes.

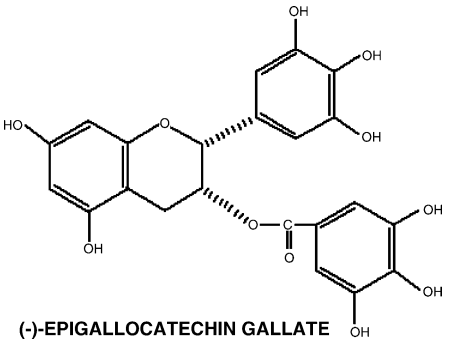
Keywords: EGCG; HL-60 cells; Apoptosis; cDNA chip; RT-PCR; Gene differential expression; Tea polyphenol

1. Introduction

(−)-Epigallocatechin gallate (EGCG) is one of the chief active compounds, polyphenols, from green tea, which scavenges free radicals, prevents lipid peroxidation and enhances physiological functions [1]. A previous study revealed that EGCG induced apoptosis on human acute promyelocytic leukemia cell line HL-60 cells [2], but their molecular mechanisms were not clarified. The gene chip technique [3] can be used to analyze the gene expression alterations within the cells with low cost, high throughput and high sensitivity, and provided a useful tool in investigating the antitumorigenic molecular mechanism of EGCG. The gene expression alterations of HL-60 after EGCG treatment was screened by the H80s gene chip, and the genes that showed differential expression were subjected to bioinformatic analysis to understand their interrelationship and interactions.

* Corresponding author. Tel.: +86 731 4498102; fax: +86 731 4498102.
E-mail address: caojin@public.cs.hn.cn (J. Cao).

0027-5107/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.mrfmmm.2004.08.004



(-)-EPIGALLOCATECHIN GALLATE

2. Materials and methods

2.1. Cell culture and ECGC treatment

The HL-60 cell line was a gift from the National Hematology Laboratory of Chinese Academy of Medical Sciences. The cells were cultured in the RPMI-1640 medium containing 10% fetal bovine serum, 100 U/ml penicillin and 100 μg/ml streptomycin in a 37 °C incubator under 5% CO_2 and saturated humidity circumstance. The experimental group cultures were treated with an optimized dosage of 550 μM EGCG SIGMA for 6 h during the logarithm proliferation stage of the HL-60 cells when the cell density reached 1×10^6 to 3×10^6/ml, and another cell culture groups without EGCG treatment served as control.

2.2. Total RNA extraction

According to the manual, the total RNA was extracted using the one step Trizol® Reagent GIBCO method from both the experimental group and control group HL-60 cultures. Total RNA content and the purity was verified under UV (SHIMADZU UV-2401PC); and then analyzed by 1% formaldehyde denatured agarose gel electrophoresis.

2.3. Preparation of the gene chip

The cDNA gene chip H80s was purchased from Shanghai BioStar Ltd., which contains a total of 8398 target gene clones. These clones were amplified by a primer, with PCR fragment length of 1000–3000 bp; and the purified target gene cDNAs were dissolved in 3× SSC solution. Cartesian Pixsys7500 sampling apparatus (Cartesian) dripped the sample cDNAs that were hydrated for 2 h, dried at room temperature for 0.5 h, UV cross-linked at 65 MJ/cm and then soaked and washed with 0.2% SDS, distilled water, and 0.2% boron hydride sodium solution each for 10 min successively before being dried in air for later use.

2.4. Preparation of the fluorescent hybridization probes

The total RNA was reversely transcripted. Each 10 μl total RNA was added into the 50 μl reverse transcription system, according the method described by Schena et al. [5] to label the mRNA through reverse transcription; the mRNAs of the experimental group were labeled by Cy5-dUTP and the mRNAs of the control group by Cy3-dUTP. Both fluorescent labeled reverse transcription products and the fluorescent hybridization probes were mixed and dissolved in 20 μl hybridization solution (6× SSC, 0.2% SDS and 100 μg/ml ultrasound fragmented salmon sperm DNA) after precipitation in ice cold anhydrous ethanol.

2.5. Hybridization and rinsing

The fluorescent hybridization probe solutions were denatured at 95 °C and transfer onto slides at a dose of 2 μl/cm^2, the solution should spread evenly. The gene chips were then placed into the Araylt hybridization cassette containing 5 μl 3× SSC solution. Hybridization was at 42 °C for 18 h in an incubator, and chips were then rinsed by solution A (1× SSC, 0.2% SDS), B (0.2× SSC) and C (0.1× SSC) each 5 min successively, and then dried in air for later use.

2.6. Assessment and evaluation of the fluorescent signals

A laser cofocusing fluorescent scanning apparatus ScanArray4000 (General Scanning, Inc.) was used to assess the hybridization data. The excitation wavelength of Cy3 and Cy5 was 550 nm and the detection wavelength was 580 and 650 nm, respectively. The scanning resolving power was 5 μm and the laser strength and PMT was set at 85%. The scanning data were evaluated by using GenePix3.0 to compare the fluorescent strength ratio of the Cy3 and Cy5 signals. To define whether there were differential gene expression,

Table 1
The representative primer sequences and their lengths

Gene name	Forward primer	Reverse primer	Product length (bp)
MAP2K1	5′-CAACTTGGAGGCCTTGCAGAA-3′	5′-CCATCGCTGTAGAACGCACCAT-3′	327
BTG1	5′-CGCCGTGTCCTTCATCTCCA-3′	5′-CAATCCGCTGTGCTGCCTGT-3′	212
KIAA0123	5′-GCACGAGCATCTGGTGGACT -3′	5′-TGCCTGTTGAGCACGTTGAG-3′	336
TREX2	5′-TTACAGCCTCGGCAGCCTCT -3′	5′-ATCAGGCGGCAAGTACATGG-3′	184
β-Actin	5′-TGGCACCACACCTTCTACAA-3′	5′-GGAAGGAAGGCTGGAAGAGT-3′	544

either the Cy5 or Cy3 fluorescent strength from the experimental or the control group should exceed 1000 and be at least twofold different. The fluorescent strength ratio of Cy5/Cy3 >2 was defined as up-regulated, and Cy5/Cy3 < 0.5 as down-regulated.

2.7. The reverse transcription polymerase chain reaction (RT-PCR)

2.7.1. RT-PCR reagents and the design and synthesis of primers

The RT-PCR reagents were purchased from Promega Co. The primer sequences were designed according to the GenBank sequences and used the primer design software and β-actin was used as the internal standard reference. The actual primer sequences were listed in Table 1. All the primers were synthesized by the Shanghai Biotech Bioengineering Services Ltd.

2.7.2. Performing the RT-PCR experiments

The experiments were performed strictly according to the manual provided. A 10 μl total RNA was immediately placed on an ice bath after denaturation at 70 °C for 10 min. A 1.0 μl 50 ng/μl Oligo-$dT_{12–18}$, 1.0 μl 10 mM dNTP, 4.0 μl 5× RT buffer, and 1.0 μl 5× 10^6U/l AMV reverse transcripase were added and the reaction system volume was adjusted to 20 μl. The mixture was remained at room temperature for 10 min, and incubated at 37 °C for 30 min, kept at 95 °C for 5 min, and again incubated at 4 °C for 5 min. 8 μl of the above product was taken for PCR amplification in the following system 4.5 μl 10× PCR buffer 1.0 μl 10 mM dNTP, each 50 pM of the upper and downstream primers 1.0 μl 5U/μl Taq enzyme; adjust the total volume of the reaction system to 50 μl. Denatured at 94 °C for 2 min in a PE9600 amplification apparatus ABI US and treated at 94 °C for 45 s, 55 °C for 45 s, 72 °C for 45 s, altogether 40 cycles; and extended at 72 °C for 5 min. The PCR amplification product was then electrophoresed on 2% agarose gel at 80 V for 60 min, ethidium bromide stained and observed and evaluated under the gel imaging system BioRad 2000, US.

3. Results

3.1. The extracted total RNA

Total RNA *OD260*/*OD280* values of the experimental and the control group were in the range 1.869–1.990 and the concentration was 75.2–97.5 μg/μl, and electrophoresis confirmed that the RNA obtained was highly purified, see Fig. 1.

3.2. The results of gene chip hybridization and their differential expression

The gene chip scanning superimposition and dispersed plots are shown in Fig. 2.

Among the two parallel gene chip hybridization evaluations of the experimental and control group, the first one revealed 175 differentially expressed genes,

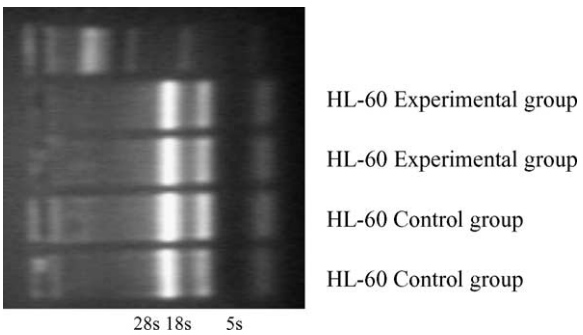


Fig. 1. The formaldehyde denatured agarose gel electrophoresis of the extracted total RNA.

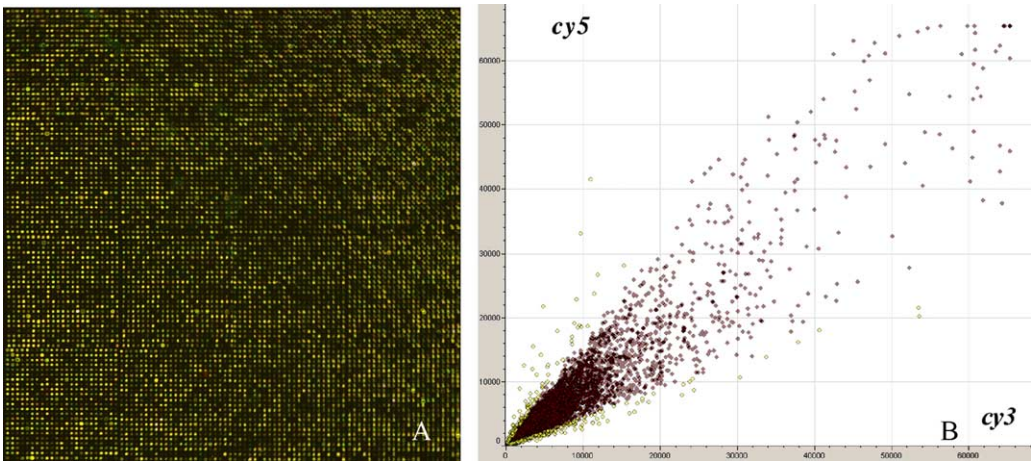


Fig. 2. The gene chip scanning superimposition (Panel A), for superimposed signals at each point. If the Cy3 signal is stronger, this indicates that the gene is down-regulated, and it appears green; if the Cy5 signal is stronger then the gene is up-regulated, and it appears red; if both the Cy3 and Cy5 signal have similar strength, it appears yellow. The dispersed plots are shown in Panel B.

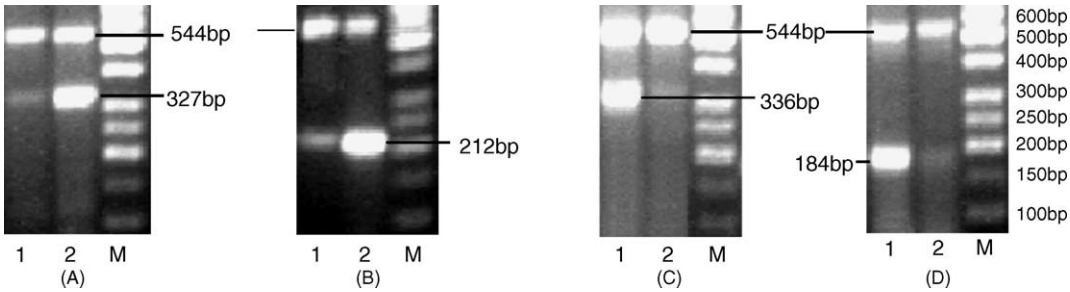


Fig. 3. Agarose gel electrophoresis of the PCR products after reverse transcription. Panel A: *MAP2K1*, Panel B: *BTG1*, Panel C: *KIAA0123*, Panel D: *TREX2*. (1) Represents the control; (2) the treated and (M) the Gene Ruler 50 bp DNA ladder.

120 were down-regulated and 55 up-regulated; while the second one revealed 238 differential expressions, 163 down regulated and 75 up-regulated. In a comparative study of the data obtained from the two parallel experiments, 97 showed a consistently similar extent of differential expression; and 58 were up-regulated, accounting for 59.8% among the differentially expressed genes 39 were down-regulated and accounted for 40.2%, see Table 2.

3.3. Validation study with RT-PCR technique

Data obtained from the gene chip screening assessment revealed genes that demonstrated differential expression. RT-PCR technique was used to validate in four randomly selected genes. The results were consistent with the data obtained from the cDNA gene chip data, the representative genes *KIAA0123* and *TREX2* were significantly expressed at lower levels in the experimental group, while the *MAP2K1* and *BTG1* significantly higher, see Fig. 3.

4. Discussion

It was well known that EGCG induces apoptosis of the human leukemia cell line cells HL-60 [2], and the apoptosis of HL-60 cell is closely related to retardation of G_1 stage cell growth [4], but the molecular mechanism was not clear. Our present experiment used the

Table 2
The consistently differential expressed 97genes

	Gene ID	GenBank ID	Gene symbol	Cy5/Cy3		
				Ratio 1	Ratio 2	Average ratio
1	2963f03	D50913	KIAA0123	0.368	0.227	0.298
2	1374e05	NM_014939	KIAA1012	0.301	0.322	0.312
3	3812c09	NM_031280	MRPS15	0.335	0.305	0.320
4	3031e12	NM_002337	LRPAP1	0.282	0.362	0.322
5	2468g12	NM_007205	PSMD13	0.330	0.332	0.331
6	1571d11	NM_002817	TREX2	0.349	0.322	0.335
7	2890e09	NM_002829	PTPN3	0.287	0.386	0.337
8	2971c11	NM_004461	FARSL	0.365	0.317	0.341
9	3025e03	NM_019854	HRMT1L3	0.315	0.370	0.342
10	2406d11	NM_003707	RUVBL1	0.314	0.373	0.344
11	3856h05	NM_018146	FLJ10581	0.378	0.319	0.349
12	2640g10	NM_001618	ADPRT	0.322	0.419	0.371
13	3999c04	AK025783	FLJ22130	0.410	0.340	0.375
14	1452b11	NM_002388	MCM3	0.381	0.384	0.382
15	1415c06	NM_003751	EIF3S9	0.360	0.413	0.386
16	3509c10	NM_006556	PMVK	0.377	0.397	0.387
17	2656a10	NM_002984	SCYA4	0.404	0.370	0.387
18	0610h05	NM_006875	PIM2	0.348	0.429	0.388
19	3839a08	NM_000349	STAR	0.314	0.463	0.389
20	1420b06	NM_006088	TUBB2	0.402	0.377	0.389
21	m23452	NM_002983	SCYA3	0.390	0.391	0.390
22	2170g02	NM_005993	TBCD	0.392	0.394	0.393
23	1581a01	NM_007103	NDUFV1	0.487	0.301	0.394
24	2689a09	AF036613	GTF2IP1	0.365	0.422	0.394
25	1500h09	NM_004875	RPA40	0.458	0.338	0.398
26	3025b01	NM_001640	APEH	0.456	0.343	0.399
27	3249f02	NM_017620	FLJ20011	0.403	0.396	0.399
28	3507d04	NM_016238	APC7	0.398	0.402	0.400
29	3914g02	NM_005745	DXS1357E	0.486	0.317	0.402
30	0738b09	NM_016292	TRAP1	0.475	0.340	0.408
31	0951f09	NM_003564	TAGLN2	0.418	0.399	0.409
32	0776e11	NM_005726	TSFM	0.451	0.372	0.412
33	3485g03	NM_004710	SYNGR2	0.453	0.375	0.414
34	m15800	NM_002371	MAL	0.416	0.416	0.416
35	3034b03	NM_002808	PSMD2	0.488	0.347	0.418
36	3108b11	D00099	NA,K-ATPase	0.497	0.339	0.418
37	3337b10	NM_005777	RBM6	0.463	0.376	0.419
38	2803b08	BC013590	MGC	0.388	0.451	0.419
39	3036g10	NM_005720	ARPC1B	0.389	0.468	0.428
40	1297a02	NM_002804	PSMC3	0.383	0.497	0.440
41	2423e02	NM_012111	C14orf3	0.444	0.440	0.442
42	1303a01	NM_001960	EEF1D	0.435	0.457	0.446
43	1566b10	NM_000365	TPI1	0.411	0.484	0.448
44	1631a03	AB023142	KIAA0925	0.426	0.470	0.448
45	3402b02	NM_004890	SPAG7	0.397	0.500	0.448
46	3654d12	NM_020040	TUBB4Q	0.422	0.481	0.452
47	1461g01	NM_006429	CCT7	0.452	0.452	0.452
48	0766b03	NM_014760	KIAA0218	0.446	0.464	0.455
49	3904g05	NM_018060	FLJ10326	0.434	0.481	0.458
50	1889g08	NM_001536	HRMT1L2	0.499	0.422	0.460
51	3726b02	NM_031266	HNRPAB	0.482	0.453	0.467
52	1656e05	NM_002778	PSAP	0.488	0.460	0.474

Table 2 (*Continued*)

	Gene ID	GenBank ID	Gene symbol	Cy5/Cy3		
				Ratio 1	Ratio 2	Average ratio
53	1096g04	NM_001100	ACTA1	0.491	0.459	0.475
54	3925b10	NM_031311	CPVL	0.497	0.457	0.477
55	3914c09	NM_006872	ALF	0.475	0.480	0.477
56	0019e12	NM_001069	TUBB	0.486	0.488	0.487
57	u23765	NM_001188	BAK1	0.485	0.496	0.490
58	1237a07	NM_004966	HNRPF	0.497	0.498	0.497
59	0693f12	AB018305	KIAA0762	2.053	2.031	2.042
60	3613e09	NM_021178	HEI10	2.100	2.051	2.075
61	4197e06	NM_005794	HEP27	2.095	2.194	2.145
62	2972b05	AL050005	DKFZp564A153	2.141	2.150	2.145
63	1555h12	NM_005868	BET1	2.015	2.315	2.165
64	1201f09	No genebank ID		2.046	2.294	2.170
65	2491c06	NM_000236	LIPC	2.074	2.297	2.185
66	1769f02	AK057236	FLJ32674	2.369	2.062	2.216
67	0911e02	NM_000700	ANXA1	2.157	2.299	2.228
68	1484b10	NM_006472	TXNIP	2.240	2.219	2.230
69	2805g02	NM_014822	SEC24D	2.271	2.242	2.256
70	0282b04	NM_004103	PTK2B	2.191	2.351	2.271
71	1181b06	NM_002394	SLC3A2	2.335	2.210	2.273
72	2598f09	AL136571	DFKZp761G2423	2.342	2.261	2.301
73	1820e05	AK057661	FLJ33099	2.278	2.438	2.358
74	3598a07	NM_003512	H2AFL	2.015	2.715	2.365
75	0973h01	NM_006475	OSF-2	2.280	2.466	2.373
76	1005d09	NM_017488	ADD2	2.358	2.452	2.405
77	3219g08	NM_007114	TMF1	2.524	2.352	2.438
78	2494h09	AV725075	AV725075	2.415	2.475	2.445
79	3748a03	BC009313	MCG	2.341	2.624	2.482
80	2869b12	NM_013286	HUMAGCGB	2.516	2.515	2.516
81	1186c12	NM_004354	CCNG2	2.741	2.298	2.520
82	3063b08	NM_003244	TGIF	2.421	2.626	2.524
83	2592c06	NM_018976	SLC38A2	2.604	2.509	2.556
84	2859b05	NM_000311	PRNP	2.561	2.663	2.612
85	m74178	NM_020998	MST1	2.839	2.598	2.719
86	1299h11	NM_005746	PBEF	3.008	2.462	2.735
87	3083c11	NM_005384	NFIL3	2.643	3.202	2.923
88	2839g01	BC019024	IMAGE	2.999	2.915	2.957
89	4959f12	NM_022476	FTS	3.327	2.612	2.969
90	1441e04	NM_016947	G8	2.848	3.201	3.024
91	m60974	L24498	gadd45	3.496	3.339	3.417
92	0679a09	AB033080	KIAA1254	3.850	3.656	3.753
93	0713a02	NM_000020	Acvrl1	4.002	3.655	3.828
94	4205e04	NM_022818	MAP1A/1BLC3	4.581	3.506	4.043
95	1087d01	BC020235	MGC	4.276	3.970	4.123
96	1209d06	NM_001731	BTG1	4.865	5.262	5.064
97	0693g07	NM_002755	MAP2K1	5.910	5.736	5.823

cDNA chip technique to investigate the EGCG induced HL-60 cell apoptosis. It discovered differential expression of 97 genes, which were closely associated with cell cycle regulation, DNA replication, DNA synthesis, transcription and translation, and ultimately resulted in cell apoptosis.

Of particular importance was the discovery of up-regulated expression of the growth arrest and DNA

damage gene *GADD45*, which is an apoptosis promoting gene and its' product is a negative regulatory factor of the G_1/S phase check point that containing binding sites to p53 [5] known to confer DNA damage responsiveness, a downstream activated product. The *GADD45* is activated when the DNA is damaged directly or up-regulated via the p53 pathway [6]. Activation, or up-regulation of *GADD45* increases its expression leading to retardation of G_1 stage cells, waiting for repair of the damaged DNA; and the apoptosis program would initiate automatically when the DNA is unable to repair. This interpretation is in accordance with the previous report of Weireb on neuroblastoma [7]. Other genes related to G_1 stage cell growth arrest were the *ACVR11* and *cyclinG2* genes. We found that the *ACVR11* gene was up-regulated in expression in our experiment. Its product is the TGF-βtype I receptor, which leads to arrest of cell growth in the G_1 stage and in turn inhibits cell cycle and enhances cell apoptosis [8]. While the product of *cyclinG2* affects growth modulation and reversely regulates cell cycle, increased expression of *cyclinG2* induced by EGCG might lead to G_1/S phase cell cycle arrest [9]. *Bak1* gene, a member of the *bcl-2* gene family, and its product promotes cell apoptosis [10], but the expression was down-regulated after EGCG treatment in our experiment. This warrants further investigation.

Up-regulation of *MAP2K1* gene expression was most prominent in our present experiments, and this result was consistently confirmed by RT-PCR test. The *MAP2K1* gene encodes MAPK kinase, which activates JNK and the JNK signaling pathway [11]. Furthermore, it has already been demonstrated that EGCG enhanced cell apoptosis via activation the JNK signaling pathway [12]. Chen et al. found that EGCG activated MAPK during the early phase of treatment, and induced cell apoptosis via JNK signaling pathway if the dose was high enough [13]. Both our DNA chip and RT-PCR experiment demonstrated that the dose of 550 μM induced cell apoptosis, which might be the same mechanism via the JNK pathway.

The other differentially expressed genes involved DNA replication and transcription and protein translation, which suggest that the EGCG induced HL-60 cell apoptosis is a result of integration of multiple genes involved within this progressive process, such as *ADPRT*, *TSFM*, *PSMID2*, *PTK2B* and others. Among these, the down-regulation of the *pim-2* gene might have particular significant importance. *Pim-2* was highly expressed in diverse tumor cells, which rendered these tumor cells resistant to most of the apoptosis inducing agents [14], and the EGCG induced down-regulation might introduce it as an adjuvant remedy in chemotherapy and radiotherapy.

Chen et al. reported [15] that arsenic oxide (As_2O_3) was a HL-60 apoptosis inducer. A cDNA chip study indicated that the arsenic oxide exerted its effects through the cell cycle regulatory related genes [16]. However, arsenic oxide is a heavy metallic compound that possesses severe nonspecific cytotoxicity to diverse cell types whether they are normal or malignantly transformation.

A possible mechanism of capase-3 activation during carotenoid-induced apoptosis in tumor cells in human leukemia (HL-60), colon adenocarcinoma (HT-29) as well as melanoma (SK-MEL-2) cell lines was reported [17]. This activation is dose dependent and follows that of caspase-8 and caspase-9 with concomitant decrease in the anti-apoptotic protein Bcl-2 and an increase in the cleaved form of BID (t-BID), and NF-kB activation is involved.

EGCG is a natural product of green tea. Studies had demonstrated that it does not induce apoptosis among the normal cells [18]. This observation could be exploited in tumor therapy. Our present study using cDNA chips investigated the gene expression spectrum of EGCG induced HL-60 cell apoptosis, to reveal a number of genes were involved. However, this is only a preliminary study, and the mechanism of these candidate genes in the induction of HL-60 cell apoptosis warrants further in depth investigation.

References

[1] Y.Z. Fang, C.P. Sun, X.H. Tian, J.H. Cong, Effect of lu-duo-wei on scavenging superoxide and hydroxyl radicals in vitro, Am. J. Chin. Med. (1998) 26.
[2] Y. Zhao, J. Cao, H. Ma, J. Liu, Apoptosis induced by tea polyphenols in HL-60 cells, Cancer Lett. 121 (1997) 163–167.
[3] O. Ermolaeva, M. Rastogi, K.D. Pruitt, G.D. Schuler, M.L. Bittner, Y. Chen, et al., Data management and analysis for gene expression arrays, Nat. Genet. 20 (1998) 19–23.
[4] A. Khafif, S.P. Schantz, T.C. Chou, D. Edelstein, P.G. Sacks, Quantitation of chemopreventive synergism between (−)-epigallocatechin-3-gallate and curcumin in normal, premalignant and malignant human oral epithelial cells, Carcinogenesis 19 (1998) 419–424.

[5] M.C. Hollander, I. Alamo, J. Jackman, M.G. Wang, O.W. McBride, A.J. Fornace Jr., Analysis of the mammalian gadd45 gene and its response to DNA damage, J. Biol. Chem. 268 (1993) 24385–24393.
[6] Q. Zhan, K.A. Lord, I. Alamo Jr., M.C. Hollander, F. Carrier, D. Ron, et al., The gadd and MyD genes define a novel set of mammalian genes encoding acidic proteins that synergistically suppress cell growth, Mol. Cell. Biol. 14 (1994) 2361–2371.
[7] O. Weinreb, S. Mandel, M.B. Youdim, cDNA gene expression profile homology of antioxidants and their antiapoptotic and proapoptotic activities in human neuroblastoma cells, FASEB J. 17 (2003) 935–937.
[8] S. Lamouille, C. Mallet, J.J. Feige, S. Bailly, Activin receptor-like kinase 1 is implicated in the maturation phase of angiogenesis, Blood 100 (2002) 4495–4501.
[9] D.A. Bennin, A.S. Don, T. Brake, J.L. McKenzie, H. Rosenbaum, L. Ortiz, et al., Cyclin G2 associates with protein phosphatase 2A catalytic and regulatory B′ subunits in active complexes and induces nuclear aberrations and a G1/S phase cell cycle arrest, J. Biol. Chem. 277 (2002) 27449–27467.
[10] K. Ory, J. Lebeau, C. Levalois, K. Bishay, P. Fouchet, I. Allemand, et al., Apoptosis inhibition mediated by medroxyprogesterone acetate treatment of breast cancer cell lines, Breast Cancer Res. Treat. 68 (2001) 187–198.
[11] J.A. Witowsky, G.L. Johnson, Ubiquitylation of MEKK1 inhibits its phosphorylation of MKK1 and MKK4 and activation of the ERK1/2 and JNK pathways, J. Biol. Chem. 278 (2003) 1403–1406.
[12] C. Chen, G. Shen, V. Hebbar, R. Hu, E.D. Owuor, A.N. Kong, Epigallocatechin-3-gallate-induced stress signals in HT-29 human colon adenocarcinoma cells, Carcinogenesis 24 (2003) 1369–1378.
[13] C. Chen, R. Yu, E.D. Owuor, R. Hu, A.N. Kong, Activation of antioxidant-response element (ARE), mitogen-activated protein kinases (MAPKs) and caspases by major green tea polyphenol components during cell survival and death, Arch. Pharm. Res. 23 (2000) 605–612.
[14] J.C. Rathmell, C.J. Fox, D.R. Plas, P.S. Hammerman, R.M. Cinalli, C.B. Thompson, The serine/threonine kinase Pim-2 is a transcriptionally regulated apoptotic inhibitor, Genes Dev. 17 (2003) 1813–1816.
[15] G.Q. Chen, J. Zhu, X.G. Shi, J.H. Ni, H.J. Zhong, G.Y. Si, et al., In vitro studies on cellular and molecular mechanisms of arsenic trioxide (As_2O_3) in the treatment of acute promyelocytic leukemia: As_2O_3 induces NB4 cell apoptosis with down-regulation of Bcl-2 expression and modulation of PML-RAR alpha/PML proteins, Blood 88 (1996) 1052–1061.
[16] J.S. Jia, S.R. Xu, J. Ma, C.R. Jia, Y.R. Yao, Y. Wang, Effect of arsenic trioxide on the expression of cyclins gene in HL60 cells (CH), Zhonghua Nei Ke Za Zhi (Chin. Intern. Med.) 42 (2003) 113–116.
[17] P. Palozza, S. Serini, A. Torsello, F. Di Nicuolo, N. Maggiano, F.O. Ranelletti, et al., Mechanism of activation of caspase cascade during β-carotene-induced apoptosis in human tumor cells, Nutr. Cancer 47 (2003) 76–87.
[18] N. Ahmad, S. Gupta, H. Mukhtar, Green tea polyphenol epigallocatechin-3-gallate differentially modulates nuclear factor kappa-B in cancer cells versus normal cells, Arch. Biochem. Biophys. 376 (2000) 338–346.