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EGCG suppresses Fused Toes Homolog protein through p53 in cervical cancer cells

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Abstract The anticarcinogenic actions of epigallocatechin-3-gallate (EGCG), one of the main ingredients of green tea, against various cancer types including cervical cancer are well documented. Studies pertaining to the exact molecular mechanism by which EGCG induces cancer cell growth inhibition needs to be investigated extensively. In the present study, we observed a stupendous dose dependent reduction in the protein expression of Fused Toes Homolog (FTS) after treatment with EGCG at 1, 10, 25 and 50 μM. Further, we were interested in finding out whether the decrease in the protein expression of FTS was due to decreased mRNA synthesis. Real time reverse transcriptase polymerase chain reaction results revealed a similar dose dependent reduction in the FTS mRNA after EGCG treatment. Chromatin immunoprecipitation analysis revealed the interaction between p53 and the promoter region of FTS. A dose dependent increase in this interaction was evidenced at 25 and 50 μM EGCG treatment. p53 silencing increased the expression of FTS and also decreased the reduction in the levels of FTS expression after EGCG treatment. The decrease in the levels of FTS was more significant at 25 and 50 μM and is associated with reduced physical interaction of FTS with Akt, phosphorylation of Akt and survival of HeLa cells. Collectively, these results conclude that EGCG induced anti-proliferative action in the cervical cancer cell involves reduced mRNA expression of FTS through p53.

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Introduction

Cervical cancer is the second most common cancer among women up to 65 years of age and is the most frequent cause of death from gynecological cancers worldwide. A woman's risk of developing cervical cancer by 65 years of age ranges from 0.69 % in developed countries to 1.38 % in developing countries [1]. Cervical cancer can be prevented and if detected early, it is generally curable. However, the treatment for metastatic or recurrent cervical carcinoma are poorly effective and with serious adverse effects [2]. These studies warrant and underscore the identification and investigation of drugs for cervical cancer treatment with minimal or no side effects. Research focused on natural compounds, particularly dietary components showing prevention and therapeutic properties in the pathogenesis of cancer have received much attention. The use of dietary compounds for cancer prevention and therapy could be of major importance because in addition to the diverse biological effects, they have low toxicity and fewer side effects than traditional chemotherapeutic agents. Many of the current anticancer drugs are natural products or derivatives thereof, highlighting the utility of natural products in drug discovery. Green tea has been shown to have cancer preventive activity in a variety of organ sites in animal models and humans. Compared with other teas, green tea has a higher catechin concentration, which is mainly attributed to its health promoting effect [3]. Among the green tea constituents, EGCG is the most abundant and active constituent in inhibiting experimental carcinogenesis. EGCG has been shown to inhibit several critical signal

transduction pathways [4]. Potential mechanisms by which EGCG exerts anticancer effects include inhibition of receptor tyrosine kinase activity [5–7], downregulation of cyclo-oxygenase 2 and activator protein 1 [5, 8], activation of p53 tumor suppressor [9] and suppression of telomerase activity [10, 11]. Treatment with EGCG prevented the carcinogenesis of cervical cancer, induced apoptosis and inhibited telomerase activity [12, 13]. EGCG also inhibited the growth of squamous cervical cancer cell lines through the induction of apoptosis [12]. Although many mechanisms for the anticancer activities of EGCG have been proposed, it is unclear which molecular events are responsible for its cancer preventive activity.

The *FTS* gene was initially identified as one of six genes deleted in a mouse mutant called Fused Toes, due to defects in limb development, and referred as FT1/FTS [14, 15]. Interaction screening analysis predicted that FTS control Akt phosphorylation by PDK1 [16]. FTS has been shown to interact with Akt through its C-terminal domain [16]. FTS is widely distributed in adult tissues [14]. Highest expression was found in kidney, testis, and brain, and lowest in spleen and liver. The wide distribution of FTS suggests that it could be a general regulator of Akt activity in the control of differentiation, proliferation and apoptosis in many cell types. Depletion of FTS by RNA interference affects the trafficking of epidermal growth factor from early to late endosome/lysosomes [17]. Recent findings from our laboratory identified the presence of FTS in the biopsy of cervical cancer patients and its expression increases with advancement of the disease [18]. In addition, suppression of this protein has resulted in the decrease in the survival of cervical cancer cells in vitro. Thus, we speculated that this protein can be targeted for effective therapy of cervical cancer. The present study is aimed to delineate the effect of EGCG on the expression of FTS in HeLa cells in vitro.

Materials and methods

Cells and reagents

Human cervical carcinoma (HeLa) cells was obtained from Korean Cell Line Bank (KCLB) and maintained in RPMI 1640 (Invitrogen Corp., Carlsbad, CA, USA) supplemented with 10 % Fetal Bovine Serum (FBS), Penicillin (100 units/ml) and Streptomycin (100 mg/ml) and incubated at 37 °C in a humidified atmosphere with 5 % CO_2. Trypsin–EDTA was used for cell harvesting and passaging. All standard culture reagents were obtained from Invitrogen. EGCG was purchased from Sigma (St. Louis, MO, USA), dissolved in DMSO and stored at −80 °C as a stock solution in aliquots. The final concentration did not exceed 0.1 % throughout the study. The primers used for the mRNA expression were obtained from Bioneer (Daejeon, Korea). Chromatin immunoprecipitation assay kit was obtained from Upstate (NY, USA). Primers for the amplication of the p53 binding site in the FTS gene were purchased from SABiosciences (Qiagen, Germantown, MD, USA).

Western blotting

The cells were washed with PBS and lysed in 200 μl of ice-cold lysis buffer (Cell Signaling Technology, Danvers, MA, USA) consisting of 20 mM Tris HCl (pH 7.5), 150 mM NaCl, 1 mM Na_2EDTA, 2.5 mM sodium pyrophosphate, 1 % Triton, 1 mM EGTA along with phosphatase and protease inhibitors. After sonication on ice, protein lysates were obtained by centrifugation at 12,000*g* at 4 °C for 10 min. The protein concentration was determined with Bradford assay reagent (Bio-Rad). Fifty μg of total protein was mixed with sample buffer (360 mM Tris HCl [pH 6.7], 60 % glycerol, and 10 % SDS). The protein samples were then heated at 95 °C for 5 min and electroseparated using 12 % sodium dodecyl sulfate—polyacrylamide gel electrophoresis (SDS–PAGE) and transferred onto a PVDF membrane (Millipore, Immobilon, MA, USA). The membrane was blocked with 0.5 % skim milk and probed with monoclonal antibody for FTS, Akt and pAkt (Santa Cruz Biotechnology, Inc., Dallas, TX, USA). The membrane was then incubated with a horseradish-peroxidase-conjugated secondary antibody and developed with WEST-ZOL Plus (iNtRON Biotechnology, Gyeonggi-do, Korea). To determine whether the amount of proteins in each lane were comparable, the membrane was stripped and probed again with a goat polyclonal antibody for β-actin (Santa Cruz).

Real time reverse transcriptase polymerase chain reaction

Total RNA was isolated from HeLa cells using Trizol (Invitrogen) and further purified with the RNeasy kit (Qiagen) according to the manufacturer's protocol. Briefly, 1 μg of RNA was reverse transcribed with reverse transcriptase at 37 °C for 30 min using Qiagen Reverse transcription kit. Real-time PCR was performed with a PCR master mix comprising 12.5 μl of 2X master mix (Qiagen), 2.5 μl of cDNA template, 2.5 μl of each FTS primer pair detailed below and 5 μl of RNase-free water. The reaction involved denaturation at 95 °C for 5 min and 40 cycles of amplification at 95 °C for 10 s, 60 °C for 10 s and 72 °C for 10 s. Real-time quantification of FTS gene was normalized to the threshold number of cycles (Ct) of

glyceraldehyde-3-phosphate dehydrogenase (GAPDH), where Ct equals the PCR cycle number at which the amount of amplified sample product reached 100 relative fluorescence units. The difference in FTS mRNA expression relative to GAPDH expression was calculated using the formulae $2^{-\Delta\Delta Ct}$: A melting curve for all products was obtained immediately after amplification to ascertain the specificity of the products. The experiment was repeated three times and the data were expressed as fold change compared with control.

Forward FTS:	CACTGGGGTGAGGCTTACTGCC
Reverse FTS:	TGGCTGCACATAGACGCCTGG
Forward p21:	TGAGCCGCGACTGTGATG
Reverse p21:	GTCTCGGTGACAAAGTCGAA GTT
Forward GAPDH:	GTCCGAGTCACCGCCTGCCG
Reverse GAPDH:	CTCGGCTGGCGACGCAAAAG

Chromatin immunoprecipitation

Chromatin immunoprecipitation (ChIP) assays were carried out using the ChIP kit from Upstate Biotechnology as described previously [19]. Briefly, soluble chromatin was prepared. Aliquots (1/10) of total chromatin DNA before immunoprecipitation were saved (input). Pre-cleared lysates were used for immunoprecipitation experiments with either p53 or IgG antibody overnight at 4 °C. The immunocomplexes were eluted by adding a 250 µl aliquot of a freshly prepared solution of 1 % SDS, 0.1 M NaHCO3. Samples were sequentially digested with RNase A (10 mg/ml) at 37 °C for 1 h and proteinase K (20 mg/ml) at 42 °C for 2 h to remove RNA and protein. The crosslinking reaction was reversed by 4 h incubation of the sample at 68 °C, and the DNA was recovered by column based elution using genomic DNA isolation kit. An aliquot of each DNA fraction was used for semi-quantitative PCR to detect the presence of specific DNA segments. The primer region spanning the p53 binding region in the promoter of FTS gene was procured from Qiagen (Cat #GPH 1078976(+)09A).

Silencing of p53 and EGCG treatment

HeLa cells were plated in antibiotic free medium and allowed to attach overnight. After attachment, the medium was replaced with transfection medium (Santa Cruz) containing small interfering RNA (siRNA) (final concentration: 50 nM) and incubated at 37 °C for 6 h. Then, the medium was replaced with regular medium and further incubated for 24 h to silence the p53 gene. The cells were further left untreated or treated with 50 µM EGCG for 24 h to assess the impact of p53 silencing on FTS mRNA and protein expression in control and EGCG treated cells using RT-PCR and Western blot analysis.

Immunoprecipitation assay

HeLa cells treated with or without EGCG (25 or 50 µM) were lysed using cell lysis buffer for 30 min on ice. After centrifugation at 12,000 rpm for 10 min at 4 °C, the supernatants were collected and 200 µg of total protein from each group was incubated with anti-Akt antibody at 4 °C overnight, followed by incubation with protein A/G agarose (Santa Cruz) for 1 h. Immunoprecipitates were washed twice with cell lysis buffer at 4 °C. Proteins bound to the beads were eluted with SDS loading buffer at 95 °C for 3 min and then subjected to SDS-PAGE and western blot analysis of FTS.

MTT assay

Cell viability was assayed using a modified colorimetric technique that is based on the ability of live cells to convert 3-[4,5-dimethythiazol-2-yl]2,5-diphenyl tetrazolium bromide (MTT), a tetrazolium compound, into purple formazan crystals with the help of mitochondrial reductases. Briefly, the cells (1×10^4/well) were exposed to different concentrations of EGCG (0, 25, 50 µM) for 24 or 48 h. At the end of the treatment, 100 µl of 0.5 mg/ml MTT solution was added to each well and incubated at 37 °C for 3 h. The formazan crystals formed were dissolved in dimethyl sulfoxide (100 µl) and incubated in dark for 1 h. Subsequently, the intensity of the colour developed was assayed using a Micro ELISA plate reader at 570 nm. The number of viable cells was expressed as the percentage of control cells cultured in serum-free medium. Cell viability in control medium without any treatment was represented as 100 %.

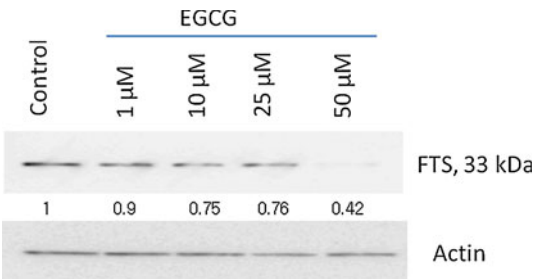

Fig. 1 Effect of EGCG on the protein expression of FTS in HeLa cells. Western blot analysis of FTS shows the reduced protein expression of FTS 24 h after EGCG treatment dose-dependently in HeLa cells. The membrane was stripped and reprobed with actin antibody to compare the protein expression

Statistical analysis

All data reported in this report were generated using in vitro assays. The significance of the observation was estimated by Student's *t* test, using data from at least three independent replicates. The observation was deemed significant if the probability of accepting null hypothesis is <0.05 (indicated by "*" in the figures).

Results

EGCG treatment reduces the protein levels of FTS

The antiproliferative actions of EGCG in cervical cancer cells are well documented. The mechanisms concerning the actions of EGCG in executing the antiproliferative effects are multiple and are still investigated in detail. In the present study, for the first time, we have shown a dose dependent reduction in the protein expression of FTS after treatment with EGCG at 1, 10, 25 and 50 μM dose levels (Fig. 1). Earlier studies in our laboratory pointed out the overexpression of FTS during the advancement of cervical cancer and have conferred radioresistance. The reduction in the protein expression supports the regulatory role of EGCG on the expression of FTS. The reduction is more prominent at 25 μM dose and almost undetectable at 50 μM.

EGCG treatment reduces the mRNA expression of FTS

We wanted to assess whether the reduction in the protein expression of FTS is due to reduced mRNA expression

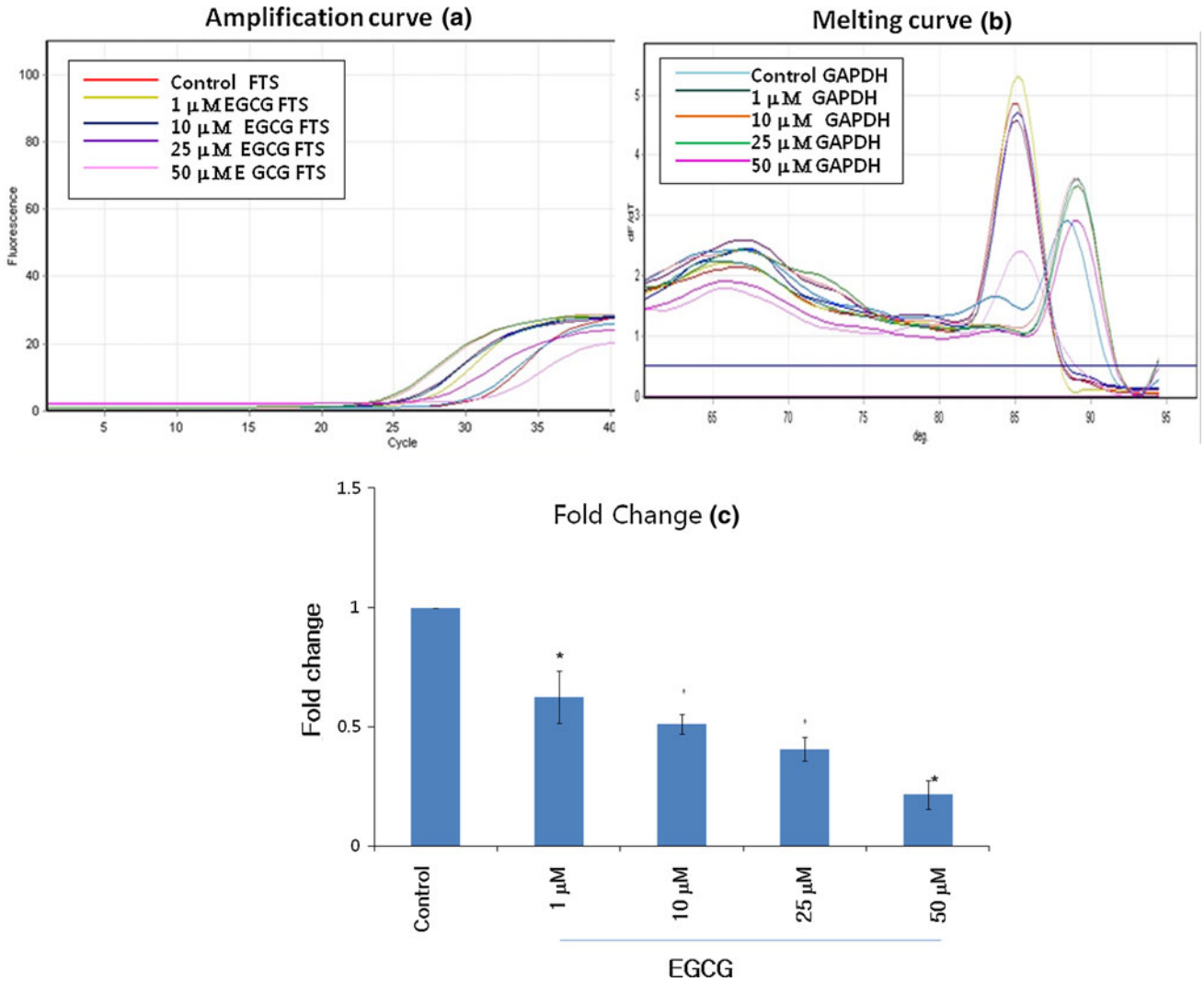

Fig. 2 Effect of EGCG on the mRNA expression of FTS in HeLa cells **a** Amplication plot, **b** Melting curve analysis showing the amplification of FTS and GAPDH genes in control and EGCG treated HeLa cells. Melting curve analysis revealed the product specificity. **c** Change in the mRNA expression was estimated and expressed as fold expression change calculated using the formulae $2^{-\Delta\Delta Ct}$. The data was represented as mean ± SEM of three independent experiments. *Asterisks* denotes statistical significance at $p < 0.05$ level

of FTS by EGCG. To test this, mRNA expression was analyzed using Real time-RT-PCR. The results obtained in the mRNA expression are similar to its protein expression. A dose dependent reduction in the mRNA expression of FTS is evidenced after EGCG treatment (Fig. 2a–c). The percentage decrease in the mRNA expression was 37, 49, 59 and 79 % after treatment with 1, 10, 25 and 50 μM of EGCG when compared with control. Transcriptional suppression of FTS by EGCG suggests that the effect of EGCG on the expression of FTS is also regulated at mRNA levels.

EGCG treatment augments the protein level of p53 and its interaction to the promoter region of FTS

In an attempt to identify the possible mechanism behind the transcriptional suppression, we have screened several factors. Among them, we have speculated that p53 could be involved in the transcriptional suppression of FTS. EGCG treatment has increased the protein level of p53. The increase in the protein level of p53 after EGCG treatment is in agreement with earlier published findings [20]. Therefore, we performed ChIP assay to assess the interaction between

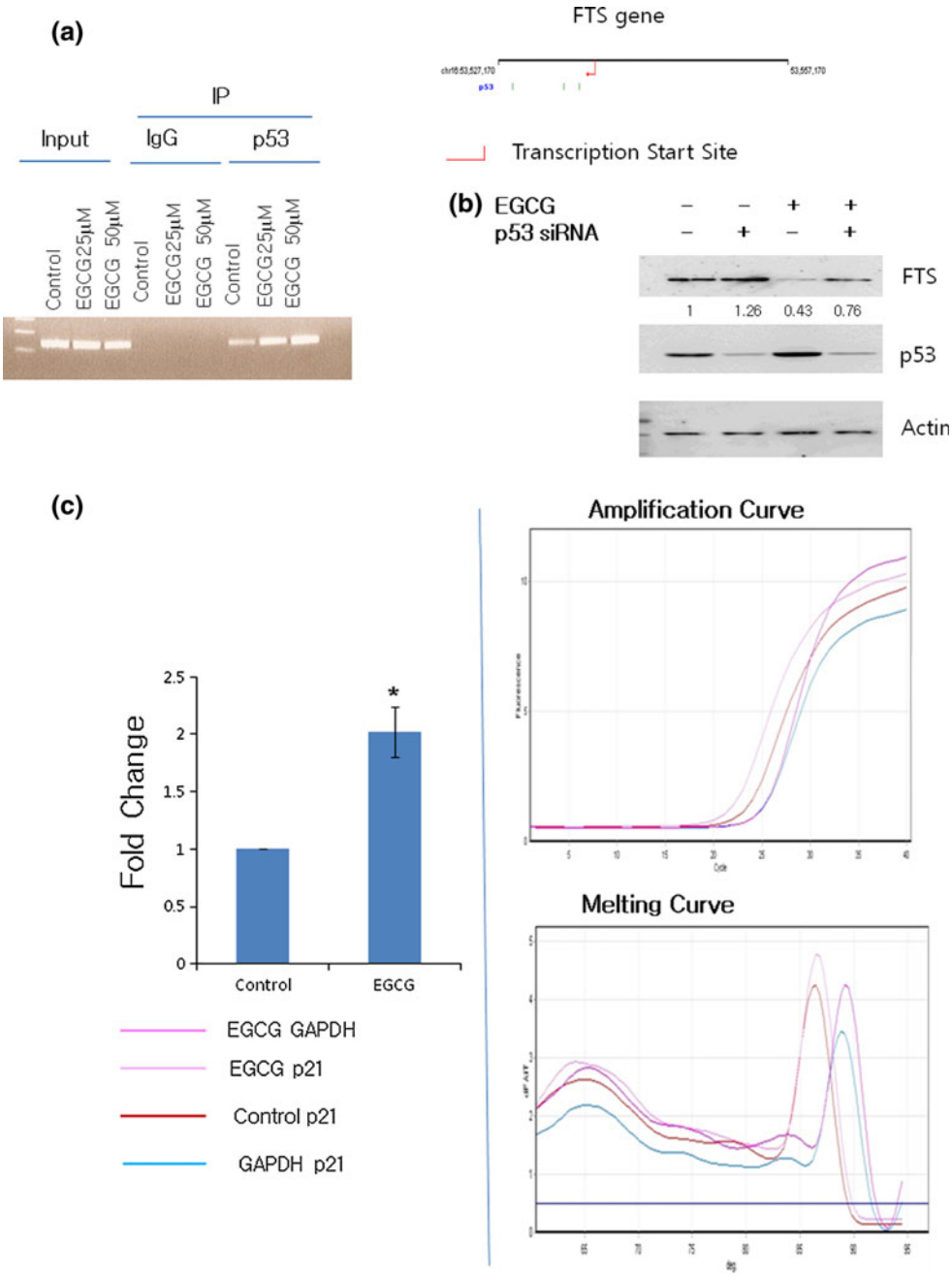

Fig. 3 **a** Effect of EGCG on the p53 binding to the promoter region of FTS in HeLa cells. **a** ChIP analysis showing the interaction between p53 (transcription factor) to the promoter region of FTS in control and EGCG treated cells after 24 h treatment. **b** Effect of EGCG (50 μM) and p53 silencing on the protein expression of FTS. EGCG treatment increased the protein levels of p53. P53 silencing increased the FTS protein expression and reduced the reduction in FTS protein expression after EGCG treatment. **c** Effect of EGCG on the mRNA expression of p21 in HeLa cells. Increase in the p53 levels after EGCG treatment is transcriptionally active. Increased mRNA expression of p21 after EGCG treatment. **d–f** Effect of EGCG and p53 silencing on the mRNA expression of FTS. p53 silencing has increased the FTS mRNA expression and reduced the reduction in FTS protein expression after EGCG treatment. *Asterisks* and *hash* denotes statistical significance at $p < 0.05$ level when compared with control and EGCG, respectively

p53 and the promoter region of FTS. For the first time, we have shown here the binding of p53 to the promoter region of FTS and this interaction is enhanced by EGCG. The increase in the binding is dose dependent (Fig. 3a).

p53 silencing and FTS expression

EGCG treatment increased the production of p53 (Fig. 3b). The increase is associated with increase in the mRNA levels of p21, a transcriptional target of p53 confirms that the increase in the levels of p53 after EGCG treatment is transcriptionally active. The levels of p21 mRNA is significantly higher in these cells when compared to control cells (Fig. 3c) suggesting that the increased p53 protein levels after EGCG treatment is transcriptionally active. Identification of p53 binding to the promoter region of FTS gene under unstimulated condition has prompted us to further investigate the role of p53 in the regulation of FTS. In this regard, p53 was silenced using siRNA based approach. p53 silencing augmented both mRNA and protein expression of FTS and also reduced the decrease in the FTS expression by EGCG. P53 silencing increased the mRNA expression of FTS by 26 % and also prevented the EGCG effects on the FTS mRNA expression by 43 % (Fig. 3c, d). Similar effects were seen in the protein expression of FTS and these highlights the involvement of p53 in the transcriptional repression of FTS by EGCG.

EGCG treatment reduces the phosphorylation of Akt and its interaction with FTS

EGCG treatment significantly reduced the mRNA and protein expression of FTS. The decrease in the expression of FTS is associated with reduced phosphorylation of Akt (Fig. 4a) in cervical cancer cells. We have also studied the physical interaction between FTS and Akt in these cells through immunoprecipitation analysis. The results have shown the presence of physical interaction between FTS and Akt and its importance in the phosphorylation of Akt in cervical cancer cells (HeLa). EGCG treatment reduced the interaction between FTS and Akt (Fig. 4b). Silencing of FTS resulted in drastic reduction in the phosphorylation of

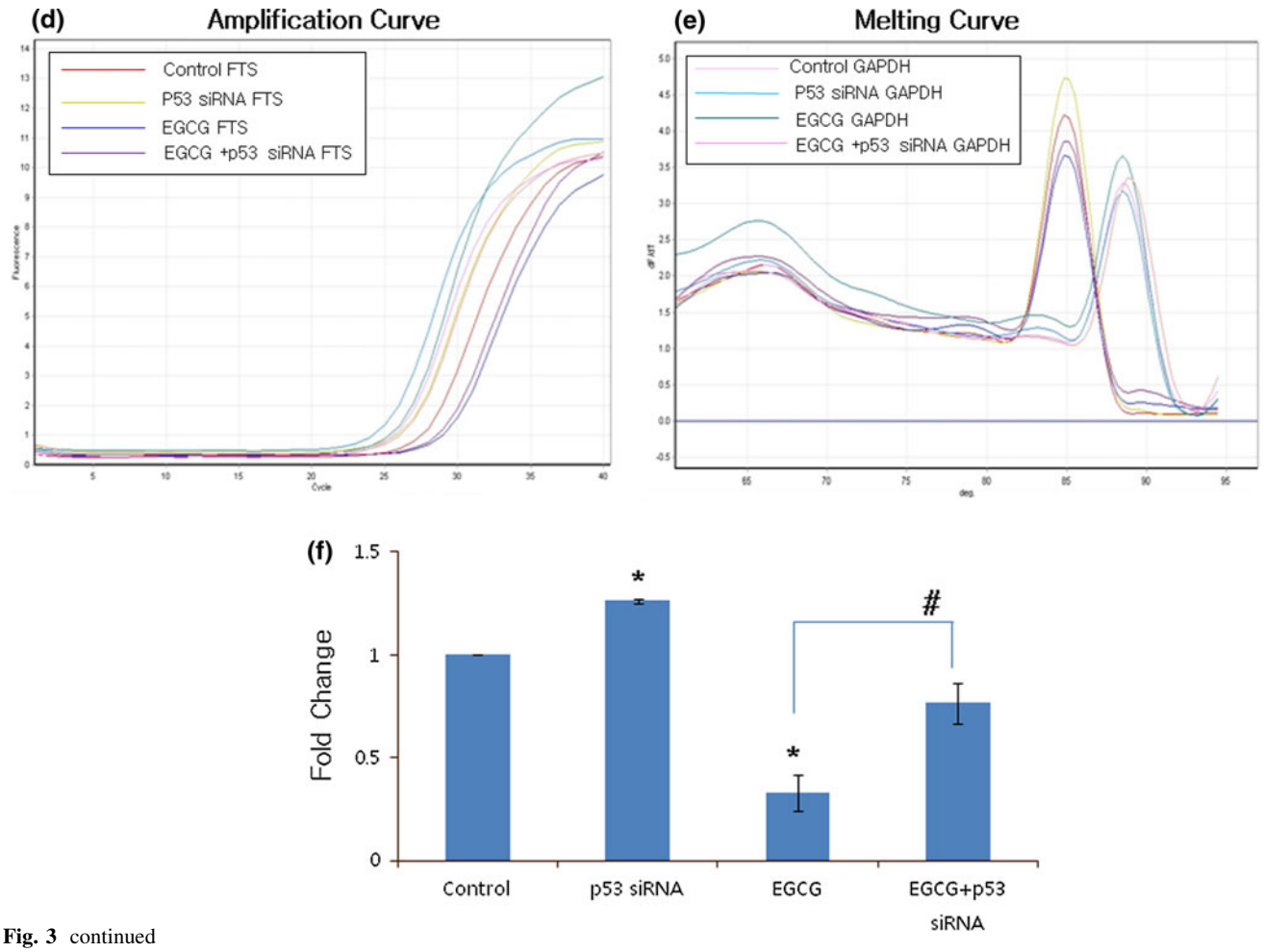

Fig. 3 continued

Akt [21]. Collectively these results suggest the functional significance of reduced FTS in EGCG treated cells.

EGCG treatment reduces the survival of cervical cancer cells

To find the functional consequence of reduced FTS expression and its interaction with Akt, survival of cervical cancer cells were evaluated after 24 and 48 h treatment with EGCG. EGCG treatment decreased the survival of HeLa cells in a time and dose dependent manner. EGCG treatment at 25 and 50 μM decreased the survival by 19.72 and 55.2 % after 24 h treatment, whereas it reduced the survival by 36.95 and 64.93 % after 48 h treatment (Fig. 5). These data suggest the requirement and importance of FTS in the survival of cervical cancer cells (Fig. 6).

Discussion

The effects of green tea, EGCG, in particular are promising in the prevention and treatment of several cancers. The mechanism by which EGCG operates in combating cancer is numerous and is an active area of research. Cervical cancer is the second most common cancer among women and it can be cured if detected early. Previous studies from our laboratory have highlighted the involvement of an oncoprotein FTS in the pathogenesis of cervical cancer [18]. The present study reports for the first time that EGCG actions in cervical cancer cells are mediated via reduced expression of FTS. The antiproliferative effect of EGCG is well documented. Silencing of FTS in cervical cancer cell line has greatly reduced the survival of these cells in vitro [17]. Silencing of this protein has also radiosensitized and accelerated the apoptosis and cell cycle arrest [21]. These findings taken together with reduced expression of FTS after EGCG treatment suggests the actions of EGCG in cervical cancer cells are mediated via decreased FTS expression. This observation has prompted us to investigate the effects of EGCG on FTS at mRNA level. Reduced mRNA expression of FTS after EGCG treatment has further strengthened the regulatory role of EGCG on FTS expression. The oncoprotein FTS has been shown to interact with Akt and increase its phosphorylation. A reduction in the phosphorylation of Akt after FTS silencing has been previously reported in our laboratory [21]. A significant reduction in the phosphorylation of Akt has been observed after EGCG in the present experiment. A similar reduction in the phosphorylation of Akt [22, 23] by

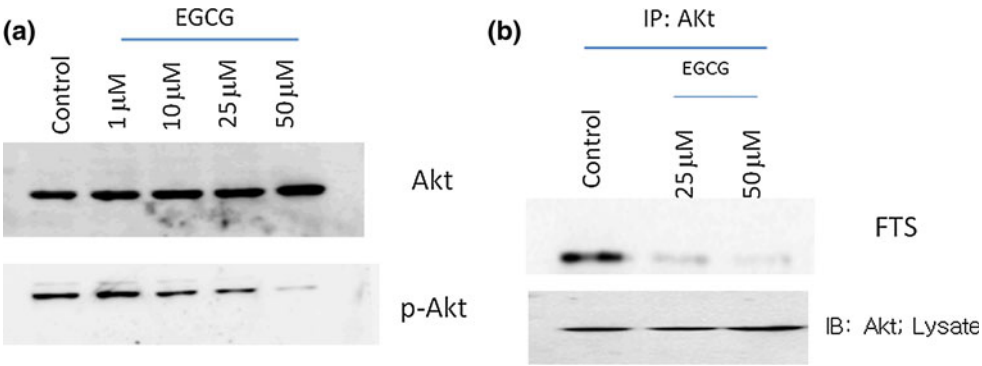

Fig. 4 Effect of EGCG on the protein expression of Akt and p-Akt in HeLa cells after 24 h. **a** EGCG treatment reduced the phosphorylation of Akt. **b** FTS physically associates with Akt in untreated cells and EGCG treatment has drastically reduced the interaction

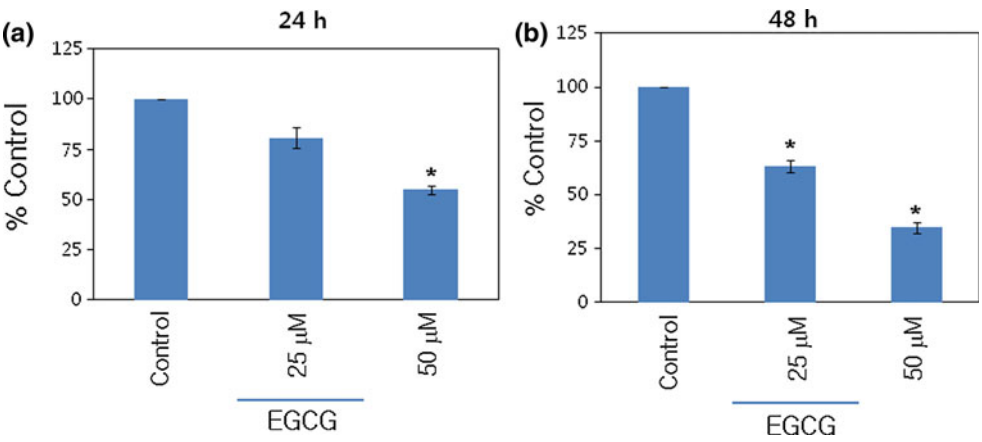

Fig. 5 Effect of EGCG on the survival of cervical cancer HeLa cells. EGCG treatment has reduced the survival of HeLa cells in vitro after 24 h (**a**) and 48 h (**b**) *Asterisks* denotes statistical significance at $p < 0.05$ level

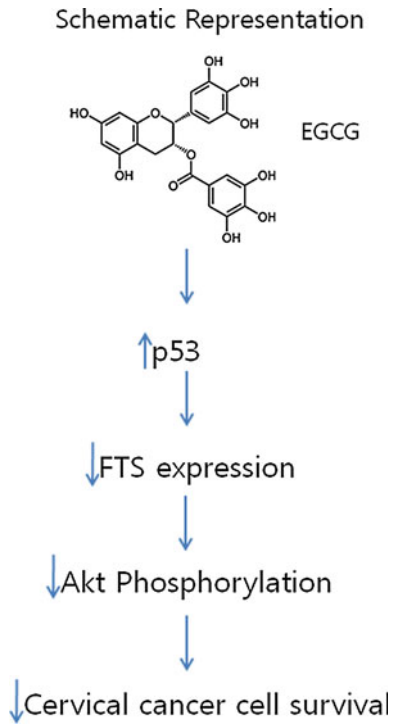

Fig. 6 Schematic representation showing EGCG induces increase in p53 and reduction in FTS expression and phosphorylation of AKT and cervical cancer cell survival

EGCG has been reported in cancer cells by other investigators. The present study also demonstrated a reduced level of p-Akt and FTS after EGCG treatment. In addition, we have shown the physical interaction between Akt and FTS in untreated HeLa cervical cancer cells and EGCG treatment greatly reduced this interaction. Previously, we have shown reduction in the phosphorylation of Akt and absence of increase in the phosphorylation after ionizing radiation (IR) in FTS silenced HeLa cells when compared to FTS intact control cells [21]. Taken together, these observations highlight the functional consequences of reduced FTS expression in EGCG treated HeLa cells.

In addition, silencing of FTS has been shown to increase caspase activity in cervical cancer cells and EGCG actions are shown to involve increased caspase activities. EGCG has been shown to inhibit epidermal growth receptor pathway. The EGCG-dependent reduction in ERK and Akt activity is associated with reduced phosphorylation of downstream substrates, including p90RSK, FKHR, and BAD. These changes are associated with increased p53, p21(WAF-1), and p27(KIP-1) levels, reduced cyclin E level, and reduced CDK2 kinase activity [24]. We have shown that FTS suppression had also reduced the EGFR downregulation, cyclin dependent kinases and increased p21 promoter activity [21]. These authors have concluded that EGCG directly inhibit ERK1/2 and Akt kinase. The present study also evidenced increased p53 protein levels and p21 mRNA suggesting that the increase in the protein expression of p53 is transcriptionally active. The present study suggests that EGCG induced suppression of Akt kinase might be mediated via reduced FTS expression. Collectively, these results point the regulatory significance of EGCG on the expression of FTS.

EGCG has been shown to alter several signal transductions, at this point time, the exact mechanism through which EGCG reduced the expression of FTS is unknown. Various studies suggest that the tumor suppressor protein p53 plays a key role in the regulation of negative cellular growth in response to EGCG [25–27]. Screening of the promoter region of FTS has shown the binding of p53, since EGCG has been shown to augment the synthesis of p53, we were interested in testing whether this could have resulted in the transcriptional repression of FTS after EGCG treatment. Our ChIP assay demonstrated the binding of p53 to the promoter region of FTS. To the best of our knowledge, this is the first report to show p53 binding to the promoter region of FTS in cervical cancer cells. A dose dependent increase in the interaction was evidenced after EGCG treatment at 10 and 25 μM. The binding was also evidenced in untreated cells. To understand the functional significance of this, we silenced the p53 gene using siRNA based approach and evaluated its effect on the mRNA and protein expression of FTS in control and EGCG treated cells. A significant increase in the expression of FTS mRNA and protein levels were noted after EGCG treatment in p53 silenced cells when compared with control cells. Further EGCG treatment induced reduction in FTS was reduced in p53 silenced cells. This is in agreement with an earlier study where treatment with EGCG resulted in activation of p53 and induction of apoptosis in prostate cancer LNCaP cells. Inactivation of p53 using siRNA rendered these cells resistant to EGCG mediated apoptosis [9]. p53 has been shown to function as transcriptional repressor that acts by activating the transcription of its target genes such as p21, PUMA, PKR, Bax, and regulate cell proliferation [28–31]. Induction of p53 expression by naringin, a plant flavonoid resulted in increased apoptosis of human cervical cancer (SiHa) cells [32]. The increase in the binding of p53 to the promoter region of FTS is associated with reduced mRNA expression suggesting that p53 is involved in the transcriptional suppression of FTS in cervical cancer cells. This interaction may have important significance and this could also be one of the potential mechanism through which p53 activating compounds bring about the antiproliferative effects in cervical cancer cells.

The data obtained in the present study clearly suggests that the antiproliferative actions of EGCG in cervical cancer cells are also mediated via reduced expression of FTS. Given the fact that cervical cancer can be cured, if detected at early stage and FTS expression increases with

advancement of this disease, the inhibitory effects of EGCG on the expression of FTS have significant implications and attest the therapeutic/preventive value of this compound in negating the process of oncogenesis. In addition to the data obtained in the present study, EGCG has numerous health promoting effects. Studies of this kind support the nutritional significance of dietary antioxidants in the treatment of cancer. Diet rich in fruits and vegetables could reduce 7–31 % of all cancer worldwide [33]. Clinical trials have indicated the relatively low toxicity and effective oral bioavailability of EGCG [34] attest the potentially ideal candidature of EGCG as antitumor agent. Emerging studies also point out the significance of p53 regulation in cancer therapy [35] and in this context the data generated in the present study suggests additional mechanism by which p53 controls tumor cell growth. The current study also warrants extensive future investigation in this area, which are currently being pursued in our laboratory.

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Conflict of interest As the corresponding author, I assure that there is no potential conflict of interest exists among the authors regarding financial and personal relationships with other people or organizations that could influence our work.

References

1. Scatchard K, Forrest JL, Flubacher M, Cornes P, Williams C (2012) Chemotherapy for metastatic and recurrent cervical cancer. Cochrane Database Syst Rev 10. doi:10.1002/14651858
2. Monk BJ, Herzog TJ (2007) The evolution of cost effective screening and prevention of cervical carcinoma: implications of the 2006 consensus guidelines and human papillomavirus vaccination. Am J Obstet Gynecol 197:337–339
3. Cabrera C, Artacho R, Gimenez R (2006) Beneficial effects of green tea—a review. J Am Coll Nutr 25:79–99
4. Shimizu M, Weinstein IB (2005) Modulation of signal transduction by tea catechins and related phytochemicals. Mutat Res 591:147–160
5. Shimizu M, Deguchi A, Hara Y et al (2005) EGCG inhibits activation of the insulin-like growth factor-1 receptor in human colon cancer cells. Biochem Biophys Res Commun 334:947–953
6. Shimizu M, Deguchi A, Joe AK et al (2005) EGCG inhibits activation of HER3 and expression of cyclooxygenase-2 in human colon cancer cells. J Exp Ther Oncol 5:69–78
7. Shimizu M, Deguchi A, Lim JT et al (2005) (-)-Epigallocatechin gallate and polyphenon E inhibit growth and activation of the epidermal growth factor receptor and human epidermal growth factor receptor-2 signaling pathways in human colon cancer cells. Clin Cancer Res 11:2735–2746
8. Jeong WS, Kim IW, Hu R et al (2004) Modulation of AP-1 by natural chemopreventive compounds in human colon HT-29 cancer cell line. Pharm Res 21:649–660
9. Hastak K, Agarwal MK, Mukhtar H et al (2005) Ablation of either p21 or Bax prevents p53- dependent apoptosis induced by green tea polyphenol epigallocatechin-3-gallate. FASEB J 19:789–791
10. Berletch JB, Liu C, Love WK et al (2008) Epigenetic and genetic mechanisms contribute to telomerase inhibition by EGCG. J Cell Biochem 103:509–519
11. Yang CS, Wang X, Lu G et al (2009) Cancer prevention by tea: animal studies, molecular mechanisms and human relevance. Nat Rev Cancer 9:429–439
12. Yokoyama M, Noguchi M, Nakao Y et al (2004) The tea polyphenol, (-)epigallocatechin gallate effects on growth apoptosis and telomerase activity in cervical cell lines. Gynecol Oncol 92:197–204
13. Noguchi M, Yokoyama M, Watanabe S et al (2006) Inhibitory effects of the tea polyphenol (-)epigallocatechin gallate on growth of adenocarcinoma cell lines. Cancer Lett 234:135–142
14. Lesche R, Peetz A, van der Hoeven F et al (1997) Ft1, a novel gene related to ubiquitin- conjugating enzymes, is deleted in the Fused Toes mouse mutation. Mamm Genome 8:879–883
15. Peters T, Ausmeier K, Dildrop R et al (2002) The mouse Fused Toes (Ft) mutation is the result of a 1.6-Mb deletion including the entire Iroquois B gene cluster. Mamm Genome 13:186–188
16. Remy I, Michnick SW (2004) Regulation of apoptosis by the Ft1 protein, a new modulator of protein kinase B/Akt. Mol Cell Biol 24:1493–1504
17. Xu L, Sowa ME, Chen J et al (2008) An FTS/Hook/p107(FHIP) complex interacts with and promotes endosomal clustering by the homotypic vacuolar protein sorting complex. Mol Biol Cell 19:5059–5071
18. Cinghu S, Anandharaj A, Lee HC et al (2011) FTS (Fused Toes Homolog) a novel oncoprotein involved in uterine cervical carcinogenesis and a potential diagnostic marker for cervical cancer. J Cell Physiol 226:1564–1572
19. Muthusami S, Senthilkumar K, Vignesh C et al (2011) Effects of Cissus quadrangularis on the proliferation, differentiation and matrix mineralization of human osteoblast like SaOS-2 cells. J Cell Biochem 112:1035–1045
20. Qiao Y, Cao J, Xie L, Shi X (2009) Cell growth inhibition and gene expression regulation by (-)- epigallocatechin-3-gallate in human cervical cancer cells. Arch Pharm Res 32:1309–1315
21. Anandharaj A, Cinghu S, Kim WD et al (2011) Fused Toes Homolog modulates radiation cytotoxicity in uterine cervical cancer cells. Mol Biol Rep 38:5361–5370
22. Zhang X, Zhang H, Tighiouart M et al (2008) Synergistic inhibition of head and neck tumor growth by green tea (-)-epigallocatechin-3-gallate and EGFR tyrosine kinase inhibitor. Int J Cancer 123:1005–1014
23. Han DW, Jung DY, Park JC et al (2010) Underlying mechanism for suppression of vascular smooth muscle cells by green tea polyphenol EGCG released from biodegradable polymers for stent application. J Biomed Mater Res A 95:424–433
24. Sah JF, Balasubramanian S, Eckert RL et al (2004) Epigallocatechin-3-gallate inhibits epidermal growth factor receptor signaling pathway. Evidence for direct inhibition of ERK1/2 and AKT kinases. J Biol Chem 279:12755–12762
25. Thakur VS, Ruhul Amin AR, Paul RK et al (2010) p53-Dependent p21-mediated growth arrest pre-empts and protects HCT116 cells from PUMA-mediated apoptosis induced by EGCG. Cancer Lett 296:225–232
26. Lee MH, Han DW, Hyon SH et al (2011) Apoptosis of human fibrosarcoma HT-1080cells by epigallocatechin-3-O-gallate via induction of p53 and caspases as well as suppression of Bcl-2 and phosphorylated nuclear factor-κB. Apoptosis 16:75–85
27. Kang SU, Lee BS, Lee SH et al (2012) Expression of NSAID-activated gene-1 by EGCG in head and neck cancer: involvement of ATM-dependent p53 expression. J Nutr Biochem. doi:10.1016/j.jnutbio.2012.07.003

28. Lee MH, Na H, Kim EJ et al (2012) Poly(ADP-ribosyl)ation of p53 induces gene specific transcriptional repression of MTA1. Oncogene 31:5099–5107
29. Vogelstein B, Lane D, Levine AJ (2000) Surfing the p53 network. Nature 408:307–310
30. Yu J, Wang Z, Kinzler KW et al (2003) PUMA mediates the apoptotic response of p53 in colorectal cancer cells. Proc Natl Acad Sci USA 100:1931–1936
31. Yoon CH, Lee ES, Lim DS et al (2009) PKR, a p53 target gene plays a crucial role in the tumor suppressor function of p53. Proc Natl Acad Sci USA 106:7852–7857
32. Elango R, Alshatwi AA (2013) Naringin induces death receptor and mitochondria mediated apoptosis in human cervical cancer (SiHa) cells. Food Chem Toxicol 51:97–105
33. Bal DG, Foerster SB, Backman DR et al (2001) Dietary change and cancer: challenges and future direction. J Nutr 131:181–185
34. Stingl JC, Ettrich T, Muche R et al (2011) Protocol for minimizing the risk of metachronous adenomas of the colorectum with green tea extract (MIRACLE): a randomised controlled trial of green tea extract versus placebo for nutriprevention of metachronous colon adenomas in the elderly population. BMC Cancer 11:360
35. Wade M, Li YC, Wahl GM (2013) MDM2, MDMX and p53 in oncogenesis and cancer therapy. Nat Rev Cancer 13:83–96