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Pathway Analysis of Genes Affected in Mcf-7 Breast Cancer Cells Treated with Recombinant Bromelain

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The contributing molecular pathways underlying the pathogenesis of breast cancer need to be better characterized. The goal of the present study is to understand the probable molecular mechanism and the associated pathway related to recombinant bromelain treatment on MCF-7 breast cancer cells. Within 1102 known genes differentially expressed to a significant degree ($p<0.001$) set, 34 genes were significantly changed between treated cells and the control cells with cutoff fold change more than 2. These genes are LYRM2, TUBB2C, LRRFIP1, HMGN2, HMGN2, GLTSCR2, RN28S1, HIST2H4B, HIST2H4A, PA2G4, ACTB, C1orf152, RPS3A, C12orf51, RAP1B, FLJ16171, CCDC59, MGEA5, KIFAP3, GPBP1, KLHDC2, TBPL1, STK38L, RIOK3, CLK4 SNORD46, C7orf60, BTG1, TMEM59, ARID4A, C6orf62, FRG1, DEFB109P1B and RBMS1. With this exploratory study using Ingenuity Pathway Analysis IPA, we aim to identify the overlapping pathways associated with recombinant bromelain treatment and its anti-cancer mechanisms in MCF-7 breast cancer cells. The pathways identification has been shown to be associated with recombinant bromelain function on cell cycle, cancer, cell death & survival, cellular development, tumor morphology, cellular growth and proliferation. This finding will enhance the power of In-vitro breast cancer study and lead to better understanding on mechanisms of action of recombinant bromelain in fighting cancer.

Key words: Cancer; cytotoxic; MCF-7; microarray; Ingenuity Pathway Analysis (IPA); bromelain.

Bromelain is an aqueous extract of pineapple that contains a complex mixture of thiol proteases and non-protease components[1]. The effects of bromelainas anti-cancer were identified on growth, invasive capacity, apoptosis and cell survival[2].In one study related to bromelain treatment of gastric carcinoma Kato III cell lines, significant reduction of cell growth was observed, while in another study bromelain reduced the invasive capacity of glioblastoma cells and reduced de novo protein synthesis[3]. Furthermore, bromelain was shown to increase expression of p53 as well as another activator of apoptosis,Bax, in mouse skin papillomas[3].The evidences of the anti-cancer activity of bromelain come from traditional remarks (in Southeast Asia) and studies of animal- and cell-based model[3]. The anti-cancer activity of bromelain is qualified mainly to its protease components[1,4]. The available commercial bromelain is obtained through tedious and costly purification method which yields bromelain at different degrees of

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purity. However, the use of recombinant bromelain is less tedious with better degree in purity of commercial bromelain. Moreover, recombinant bromelain provides substantial quantity which has resistant to natural inhibitors like pH and temperature [5]. Microarray provides expression levels for thousands of genes at the same time. The differentially expressed genes can be studied with different pathway analysis tools to connect with existing biological pathways. In this study, we used Ingenuity Pathway Analysis (IPA) and Ingenuity iReport software with the individual gene analysis by Gene Ontology (GO) analysis. These software applications provided powerful tools to understand the pathway analysis results that evaluate the microarray data from recombinant bromelain treatment on MCF-7 breast cancer cells. Thus, the recombinant bromelain treatment may exert its toxicity in relation to cancer.

MATERIALS AND METHODS

Cell culture and recombinant bromelain treatment

Human breast cancer cell line MCF-7(ATCC No: HTB-22) was obtained from American type Collection Culture and used in this study. Recombinant bromelain[6] was employed to treat MCF-7 as anti-cancer agent. Total RNA extraction was carried out to isolate total RNA of untreated and treated cells from three different batches using the RNeasy mini kit (Qiagen, Netherlands) according to the manufacturer's instructions.

Microarray analysis

Microarray analysis was carried out using the GeneChip® Gene 1.0 ST Array System for Human (Affymetrix, USA) containing total of 30,000 genes in human genome. The microarray was performed in three batches. The cDNA of each sample was synthesized from 100 ng of the total RNA using the GeneChip® cDNA Synthesis Kit (Invitrogen, USA). The cDNA was then used for expression profiling using GeneChip® Hybridization, Wash and Stain kits (Invitrogen, NY, USA). The probe array was scanned through the Affymetrix Gene Chip® Scanner 3000 7G (Affymetrix, USA) and analyzed using NetAffy Analysis Center and GeneChip® Operating Software (GCOS) (Affymetrix, USA).

Gene Expression Data analysis

Gene Ontology (GO) analysis, Ingenuity Pathway Analysis (IPA) and Ingenuity Ireport Analysis were performed to determine which functional processes were differentially represented in the combined gene lists of 1102 genes compared to the Ingenuity knowledge base. Finally, the established microarray data was used to modify a pathway that predicts the effect of recombinant bromelain anti-cancer activity on MCF-7 cells.

RESULTS AND DISCUSSION

Global view of changes in gene expression by recombinant bromelain treatment

Microarray analysis shows the functional for simultaneous profiling of global gene expression and new genes that undiscovered or new functions of known genes[7]. In this study, microarray analysis was conducted of the global gene expressions profiles in MCF-7 cells following treatment with recombinant bromelain. The t-test showed that from the 30,000 genes in the human genome, approximately 1102 genes were significantly ($p < 0.05$) changed after the treatments. Within this gene set, 34 genes were significantly changed between the treated cells and the control cells with cut-off expression fold change ≥2.00 which 12 were up-regulated and 22 were down-regulated. The up- and down-regulated gene sets, with their functional locations and respective fold

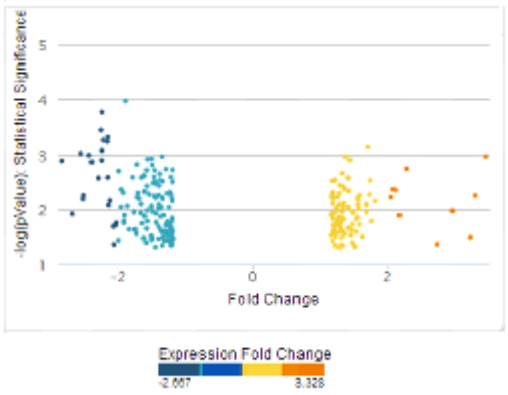

Fig. 1. Heat map of statistical significance vs. Fold Change showed the relative expression changes in MCF-7 following recombinant bromelain treatment by Ingenuity® iReport.Orange denotes up-regulation, and blue down-regulation. The data shown represent the mean values of fold changes in expression level obtained from three independent experiments

changes, were included (Table 1). Heat map of statistical significance vs. Fold Change plot showed the relative expression changes in MCF-7 following recombinant bromelain treatment (Figure1).

Table 1. List of genes affected significantly by recombinant bromelaim treatment identified by Ingenuity® iReport

Gene Symbol	P-Value	Q-Value	Regulation	Fold Change
LYRM2	0.001088	0.851975	up	3.328143
TUBB2C	0.005651	0.920447	up	3.148847
LRRFIP1	0.032381	0.977094	up	3.067804
HMGN2	0.010749	0.95575	up	2.801376
HMGN2	0.012203	0.95575	up	2.622454
GLTSCR2	0.043079	0.983883	up	2.592183
RN28S1	0.00182	0.862527	up	2.206807
HIST2H4B	0.013046	0.95575	up	2.132289
HIST2H4A	0.013046	0.95575	up	2.132289
PA2G4	0.004465	0.912169	up	2.096833
ACTB	0.004343	0.902864	up	2.060998
C1orf152	0.006044	0.920447	up	2.043241
RPS3A	0.017898	0.95575	down	-2.01332
C12orf51	0.019858	0.95575	down	-2.02315
RAP1B	0.043446	0.983883	down	-2.03774
FLJ16171	0.020783	0.955795	down	-2.04596
CCDC59	0.006977	0.935229	down	-2.08273
MGEA5	0.0085	0.95575	down	-2.09673
KIFAP3	4.90E-04	0.851975	down	-2.10551
GPBP1	0.002624	0.862527	down	-2.1059
KLHDC2	5.92E-04	0.851975	down	-2.11128
TBPL1	5.57E-04	0.851975	down	-2.15716
STK38L	1.71E-04	0.841095	down	-2.17162
RIOK3	8.56E-04	0.851975	down	-2.17181
CLK4	0.001279	0.851975	down	-2.17391
SNORD46	3.65E-04	0.851975	down	-2.18119
C7orf60	0.002688	0.862527	down	-2.2111
BTG1	0.001368	0.862527	down	-2.28457
TMEM59	0.001034	0.851975	down	-2.31284
ARID4A	0.005628	0.920447	down	-2.37987
C6orf62	0.00654	0.920447	down	-2.38592
FRG1	9.60E-04	0.851975	down	-2.41483
DEFB109P1B	0.012251	0.95575	down	-2.52359
RBMS1	0.001307	0.853522	down	-2.6674

Gene networks affected by recombinant bromelain treatment

Ingenuity Pathway software (Ingenuity® Systems,www.ingenuity.com) was performed to examine functional correlations within the different between treated and untreated cells with different periods . Data sets containing gene identifiers and corresponding expression values were uploaded into the application. Each gene identifier was mapped to its corresponding gene object in the Ingenuity Pathways Knowledge Base. Genes differentially expressed with $p<0.01$ were overlaid onto global molecular networks developed from information contained in the knowledge base. Networks were then algorithmically generated based on their connectivity. Networks were "named" on the most common functional group (s) present. Networks consider all possible direct interactions[8]. In this study, the main target was to correlate the possible modes of recombinant

bromelain actions towards its toxicity and anti-proliferation action in treating MCF-7 breast cancer cell line through differential gene expression and network analysis. It was clear from the analysis that recombinant bromelain treatment on MCF-7 cells acted differently from the untreated MCF-7 cells on the gene expression following the different networks and downstream pathways activation. By using IPA analysis (Table 2, Figure 2), this network obtained 16 scores with 19 focus molecules and its function involved in cell cycle, cancer and reproductive system disease.

Table 2. High-scoring network in MCF-7 cancer cells with recombinant bromelain treatment identified by Ingenuity® Pathway Analysis

Molecules in Network	Score	Focus Molecules	Top Functions
BTG1, CCNB1, CD2BP2, CDH1, CDH11, CDKN28,CBNPA, CITED2, ERBB2, FN1, FSTL3, GPI, HPCAL1, HSD17B8, IER3, ILK, ITGB1, JUNB, KDM5B,KLF4, MAPK13, NDE1, NEK2, PDLIM4, PFKP, PGR, PHF20, PRIM1, SAT1, SCNN1A,SDC1,SDCBP, SMAD7, TGFB1	16	19	Cell Cycle, Cancer, Reproductive System Disease
CASP8, CD44, CDKN1A, ERBB2, FOS, FOSL1, FSTL3, HIF1A, IFNG, IGF1R, IL6, IL8, JUN, JUNB, KRT7,LAMP2, LAMP2, LPAR2, MAPK13, MMP13, MUCI, PADI4, PDLIM4, PRIM1, PTEN, RELA, RHOC, SMAD2, SMAD3, STAT1, STAT, TGFB1, TWIST1, ZNF148	9	14	Cell Death & Survival, Cellular Development, Cellular Growth and Proliferation
BAX, BCL2, CARM1, CCNB1, CCNDL, CDC27,CDK2, CDK4, CDK5, CDKN1A, CDKN1B, CDKN2A, CDKN1C, CEBPD, CHTOP, CLU, CSF1,CTNB1,DNMT1, ERBB2, ESR1, FBL, FHOD1, FLII, LTF, MY, NME1, NOP58, NRG1, PGR, SOX2, TFF1, TP53, ZBED5	8	13	Tumor Morphology, Cell Cycle, Cancer

In this study, the top major network is shown in Figure 2, and was identified around the breast Cancer 1 (BRCA1) gene which is well-known gene whose mutation presents an extremely increased risk of breast cancer on carriers[9,10].BRCA1 is linked with a huge number of DNA repair pathways, especially non-homologous end joining (NHEJ) and homologous recombination. Individuals with an inherited impairment in DNA repair capability are often at increased risk of cancer[11]. According to the pathway analysis (Figure 2) the histone demethylase lysine demethylase 5b (KDM5B) was found to be affected by three up regulated genes which were SAT1, PHF20 and H5D17B8 and three down regulated genes which were CD28P2, NDE1 and SCNN1A. Thus, KDM5B is over-expressed in breast cancers case, plays role of anoncogene, represses various tumor suppress orgenes including BRCA1[12], regulates cell cycle control genes in cancer and is expressed in the early epiblast[13]. Furthermore, CCAAT/enhancer-binding protein delta (CEBPD) was found down regulated by recombinant bromelain treatment (Figure1), thus down regulated cell growth, differentiation and in promoting tumor invasiveness. It is also has direct impact on breast cancer and apoptosis of breast cell line[17]. Moreover, Cyclin-dependent kinase 4 (CDK4) was found down regulated by recombinant bromelain treatment (Figure 2), thus the pharmacological inhibition of CDK4 kinase is found to target breast cancer cells preferentially and may have efficacy in the treatment of breast cancer[14].

As shown in Figure 2, FHOD1was down regulated by recombinant bromelain treatment. FHOD1 (Formin homology 2 domain containing protein) is a mammalian form in which regulates cytoskeletal architecture. Previous study showed

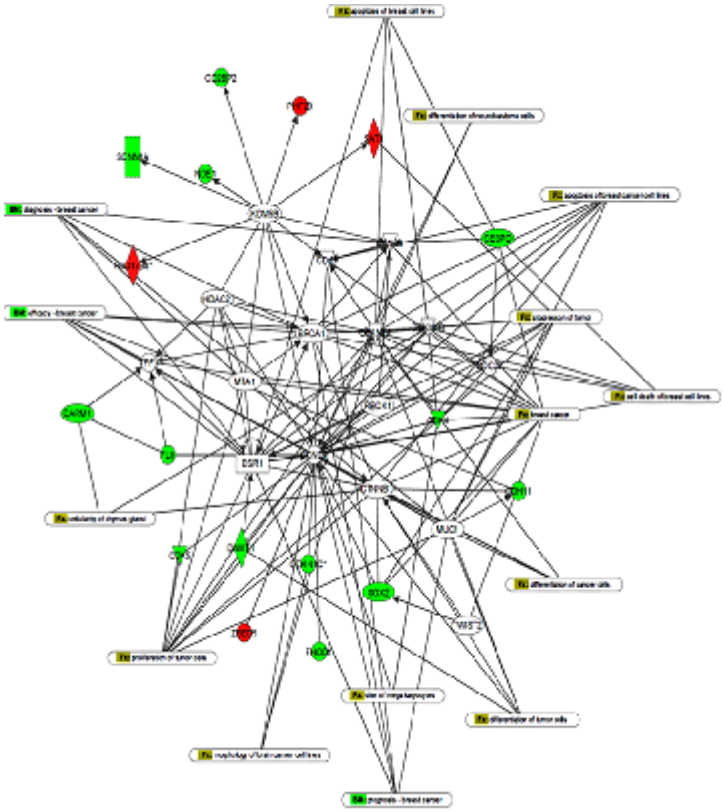

Fig. 2. The top of the major networks by differentially expressed gene set showing the connectivity of differentially expressed genes (e"1.0, t-test, p<0.001). The network consists with 13 down regulated (green) and 4 up-regulated genes(red). BM: biomarker, FX: function

that down regulation of FHOD1 can represses migration and invasion of breast cancer cells through regulation of stress fiber formation and function by modulating several downstream mediators[15]. SOX2 knockdown prevented mammosphere formation and delayed tumour formation in breast tumour initiation models. Based on the recent studies, SOX2 is up-regulated in several types of cancers including pancreatic intraepithelial neoplasia prostate cancer, gastric carcinoma, and breast cancer[16]. Moreover, other studies showed that SOX2 acts as a tumour-suppressive when it is down regulated. This is happened through inhibition the cell growth by cell-cycle arrest and apoptosis[17]. Thus, these previous reports supported that recombinant bromelain treatment might interrupt the cancer cells growth by arresting cell cycle as part of down regulated SOX2 mechanism of action and apoptosis.

The second and third networks got 9 scores with 14 focus molecules and 8 scores with 13 focus molecules, respectively (Table 2, Figure 3 and Figure 4). They are involved in Cellular growth and proliferation, cell cycle, Cancer, Cell death and survivals, and tumor morphology. The most affected genes in networks 1,2 and 3 are BTG1, FHOD1, SAT, PHF20, GLTSCR2, H5D17B8, CD28P2, NDE1, SCNN1A, CEBPD, CDK4, KLF4 and SOX2.

According to previous study[18], BTG1 is a member of a new family of anti-proliferative genes and negatively regulates cell proliferation (Table 2, Figure 3). Their finding strongly supports the regulation of the gene during our experiment. Furthermore, according to their studies, BTG1 expression was maximal in the G0/G1 phases of the cell cycle and down regulated when cells progressed throughout G1. In that case, BTG1 expression was associated with the inhibition of

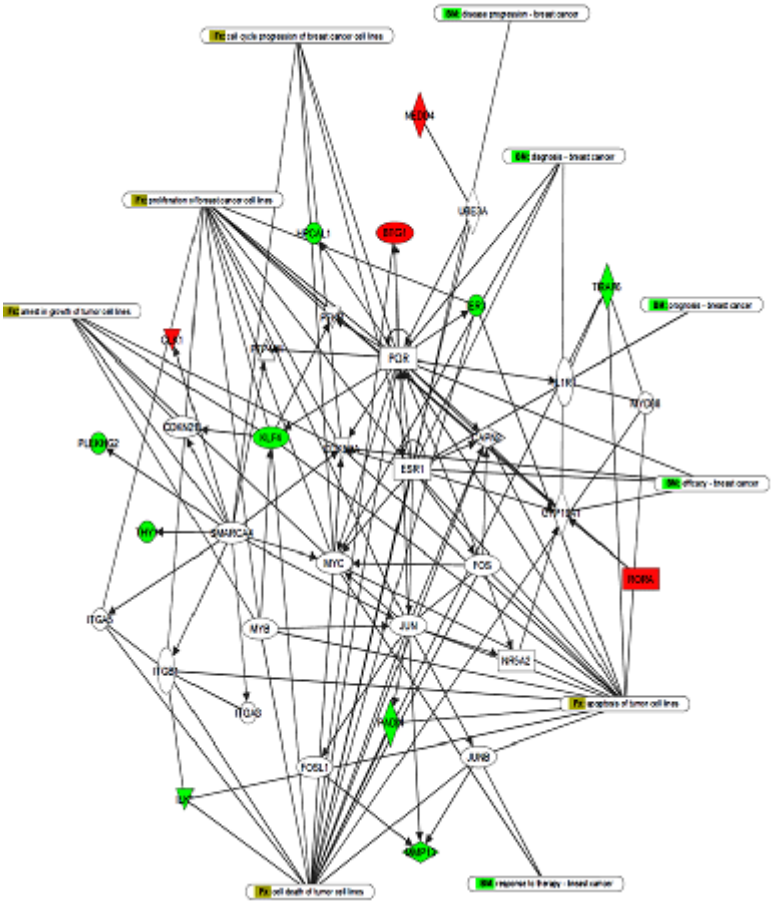

Fig. 3. The second of the major networks by differentially expressed gene set showing the connectivity of differentially expressed genes (e"1.0, t-test, p<0.001). The network consists with 9 down regulated (green) and 4 up-regulated genes (red). BM: biomarker, FX: function

proliferation where increased BTG1 levels will decrease proliferation. Another study had shown that BTG1 mediates chemotherapy-induced apoptosis in breast cancer cells[19]. Furthermore, BTG1 regulates estrogen receptor-á (Esr1) functions[20]. A recent work[21] that used the toxicogenomics (the use of gene expression profiling in toxicology) reported an attractive approach to predict toxicity and to gain a mechanistic understanding of toxic changes which also is in agreement with the changing of our genes expression after the treatment. It is noticeable that each compound may produce its own unique expression profile (signature gene expression) in toxic mechanism, even with the limited compounds set evaluated in this study which are FHOD1 and SAT1 (Table 2, Figure 2). Previous study showed that tumor progression is accompanied by significant changes in the levels of expression of SAT1 in human prostate cancer specimens[22], while the other toxicity genes are explained with their actions through the coming networks and pathways.

The most significant fact of second and third networks in our study was the implicated genes around Myc/c-Myc (Figure 3 and Figure 4). Myc is a regulator gene that codes for a transcription factor. A mutated version of Myc is found in many cancers, which causes Myc to be constitutively (persistently) expressed[23]. This leads to the unregulated expression of many genes, some of which are involved in cell proliferation, and results in the formation of cancer. Previous studies show that transforming growth factor beta

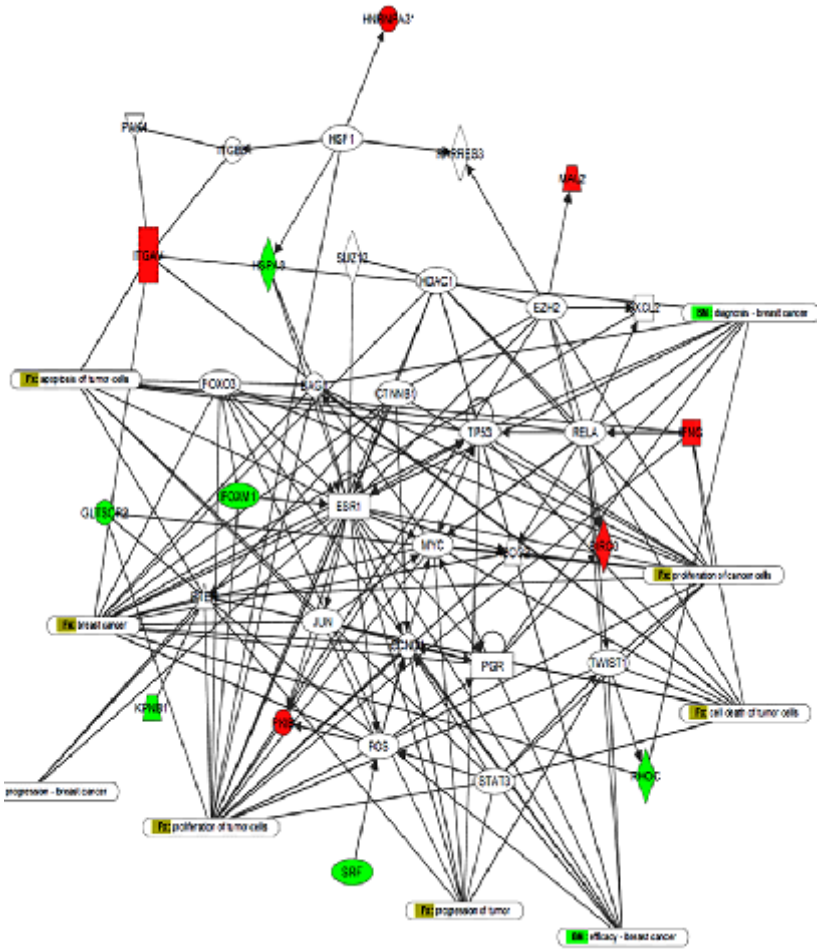

Fig. 4. The Third of the major networks by differentially expressed gene set showing the connectivity of differentially expressed genes (≥1.0, t-test, $p<0.001$). The network consists with 6 down regulated (green) and 6 up-regulated genes (red). BM: biomarker, FX: function.

1 (TGFB1) can act as a tumor suppressor by mediating growth arrest via the CDK inhibitors and by inhibiting the expression of c-Myc, CDK4, and CDC25A[24].

ESR1 (Estrogen receptor alpha) is implicated in the development of breast cancer (Figure 2, Figure 3 and Figure 4). Based on data from both clinical and animal studies, risk factors associated with breast cancer reflect cumulative exposure of the breast epithelium to estrogenin breast cancer[25]. It has been shown that between 60% to 80% of breast cancer cells in human expressed ESR1[26]. Thus, estrogen receptor regulated factor was found to be involved in breast cancer progression[27].

Gliomatumour-suppressor candidate region gene 2 (*GLTSCR2*/*PICT-1*) is frequently altered in various human tumours, including diffuse gliomas (Figure 4). GLTSCR2 has several lines of evidence, including PTEN-phosphorylating and cell-killing activities, suggests that GLTSCR2 participates in the suppression of tumour growth and development [28]. Furthermore, recent research demonstrated that GLTSCR2 involved in mitochondrial apoptotic cascades and the suppression oftumour growth and development[29]. Other important gene in network 3 (Figure 4) is FOXM1 which is the most critical cell-cycle regulator among the forehead transcription factor family, a link between ERá and transcriptional

regulation of FOXM1 in breast carcinoma, would be very crucial and would provide a novel mechanism of estrogen action in hormone-responsive cells[26]. Moreover, KLF4 (Kruppel-like factor 4) is highly expressed in more than 70% of breast cancers [30]. As shown in Figure 4, the Knockdown of KLF4 in breast cancer cells (MCF-7) decreased the proportion of stem/progenitor cells[31]. Consistently KLF4 knockdown also suppressed cell migration and invasion in MCF-7 and MDA-MB-231 cells, reduced colony formation in vitro and inhibited tumorigenesis, supporting an oncogenic role for KLF4 in breast cancer development[32]. These finding strongly supports the mode of recombinant bromelain actions toward MCF-7 breast cancer cells.

CONCLUSION

This study provided enormous information about genes expression that has a significant cutoff of more than 2 fold changes between treated cells and control cells. The pathway analysis results underline the possible routes of recombinant bromelain treatment in cell cycle, cancer, cell morphology, cellular growth and proliferation, cell death and survivals, and tumor morphology. Analysis reveals that important regulatory molecules, such as BTG1, FHOD1, SAT, PHF20, GLTSCR2, H5D17B8, CD28P2, NDE1, SCNN1A, CEBPD, CDK4, KLF4 and SOX2, play a significant role in different pathways because of their altered gene expression by recombinant bromelain treatment. Thus, recombinant bromelain treatment achieved its positive widespread effects on MCF-7 breast cancer cells as an anti-cancer agent. We believe that this study will assist in the identification of additional potential candidate markers in cancer.

ACKNOWLEDGEMENTS

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REFERENCES

1. Chobotova, K., Vernallis, A.B., Majid, F.A. Bromelain's activity and potential as an anti-cancer agent: Current evidence and perspectives. *Cancer Lett*., 2010; **290**:148-56.
2. Chobotova, K., Vernallis, A.B., Majid, F.A.A. Bromelain's activity and potential as an anti-cancer agent: Current evidence and perspectives. *Cancer Lett*., 2010;290:148-56.
3. Hale, L.P., Greer, P.K., Trinh, C.T., James, C.L. Proteinase activity and stability of natural bromelain preparations. *Intl. Immunopharmacol.,* 2005; **5**:783-93.
4. Maurer, H.R. Bromelain: biochemistry, pharmacology and medical use. *Cell Mol. Life Sci*., 2001; **58**: 1234 - 45.
5. Amid, A., Ismail, N.A., Yusof, F., Salleh, H.M. Expression, purification, and characterization of a recombinant stem bromelain from ananas comosus. *Process Biochem*., 2011;46:2232-9.
6. Bala, M., Salleh, H.M., Amid, A., Mel, M., Jami, M.S. Recovery of recombinant bromelain from *Escherichia coli* BL21-AI. *African J.Biotech.,* 2011; **10**: 18829-32.
7. Thornton, S., Sowders, D., Aronow, B., Witte, D.P., Brunner, H.I., Giannini, E.H., et al. DNA Microarray Analysis Reveals Novel Gene Expression Profiles in Collagen-Induced Arthritis. *Clin. Immunol.,* 2002; **105**:155-68.
8. Ghosh, S., Zang, S., Mitra, P.S., Ghimbovschi, S., Hoffman, E.P., Dutta, S.K. Global gene expression and Ingenuity biological functions analysis on PCBs 153 and 138 induced human PBMC in vitro reveals differential mode(s) of action in developing toxicities. *Environment International*. 2011; **37**: 838-57.
9. Miki, Y., Swensen, J., Shattuck-Eidens, D., Futreal, P.A., Harshman, K., Tavtigian, S., et al. A strong candidate for the breast and ovarian cancer susceptibility gene BRCA1. *Science,* 1994; **266**: 66-71.
10. Yoshida, K., Miki, Y. Role of BRCA1 and BRCA2 as regulators of DNA repair, transcription, and cell cycle in response to DNA damage.*Cancer Sci*., 2004; **95**: 866–71.
11. Campisi, J., d'Adda, D.I., Fagagna, F. Cellular senescence: when bad things happen to good cells. *Rev. Mol. Cell Biol.,* 2007; **8**: 729-40.
12. Thakur, R., Mishra, D. Modulation of cell death pathways in cancer stem cells: Targeting histone demethylases. *Sci. Research,* 2012; **3**: 720-30.
13. Dey, B.K., Stalker, L., Schnerch, A., Bhatia, M., Taylor-Papidimitriou, J., Wynder, C. The Histone Demethylase KDM5b/JARID1b Plays a Role in Cell Fate Decisions by Blocking Terminal Differentiation. *Mol. Cell Biol.,* 2008; **28**: 5312-27.
14. Dean, J.L., Thangavel, C., McClendon, A.K., Reed, C.A., Knudsen, E.S. Therapeutic CDK4/

6 inhibition in breast cancer: key mechanisms of response and failure. *Oncogene*. 2010; 29.

15. Jurmeister, S., Baumann, M., Balwierz, A., Keklikoglou, I., Ward, A., Uhlmann, S., et al. MicroRNA-200c Represses Migration and Invasion of Breast Cancer Cells by Targeting Actin-Regulatory Proteins FHOD1 and PPM1F. *Mol. Cell Biol.*, 2012; **32**: 633-51.
16. Chen, Y., Shi, L., Zhang, L., Li, R., Liang, J., Yu, W., et al. The Molecular Mechanism Governing the Oncogenic Potential of SOX2 in Breast Cancer.*J. Biol. Chem.*,2008; **283**:17969-78.
17. Otsubo, Akiyama, Y., Yuasa, K.Y.aY. SOX2 is frequently downregulated in gastric cancers and inhibits cell growth through cell-cycle arrest and apoptosis. *Br. J. Cancer,* 2008;98:824–31.
18. Rouault, J., Nicole, Falette., Fabienne, Guéhenneux, Céline, Guillot, R.R., Wang, Q.Berthet, C. Moyret-Lalle, C., Savatier, P., Pain, B., Shaw, P., Berger, R., Samarut, J., Magaud, J., Ozturk, M., Samarut, C., Puisieux, A., Identification of BTG2, an antiproliferative p53"dependent component of the DNA damage cellular response pathway. *Nature Genetics*,1996; **14**: 482 - 6
19. Cho., I.J., Lee, A.K., Lee, S.J., Lee, M.G., Kim, S.G. Repression by oxidative stress of iNOS and cytokine gene induction in macrophages results from AP-1 and NF-κB inhibition mediated by B cell translocation gene-1 activation. *Free Radic. Biol. Med.*,2005; **39**: 1523-36.
20. Hamatani, T., Daikoku, T., Wang, H., Matsumoto, H., Carter, M.G., Ko, M.S.H., et al. Global gene expression analysis identifies molecular pathways distinguishing blastocyst dormancy and activation. *Proceedings of the National Academy of Sciences of the United States of America*,2004;101:10326-31.
21. Blomme, E.A.G., Yang, Y., Waring, J.F. Use of toxicogenomics to understand mechanisms of drug-induced hepatotoxicity during drug discovery and development. *Toxicology Lett.*,2009;**186**: 22-31.
22. Saverio, B., Pierpaola, D., Serenella, A., Cesare, C., Bruno, M., Auro, T., et al. Tumor Progression Is Accompanied by Significant Changes in the Levels of Expression of Polyamine Metabolism Regulatory Genes and Clusterin (Sulfated Glycoprotein 2) in Human Prostate Cancer Specimens. *Cancer Research*, 2000; **60**: 28-34.
23. Li, H., Lee, T.H., Avraham, H. A Novel Tricomplex of BRCA1, Nmi, and c-Myc Inhibits c-Myc-induced Human Telomerase Reverse Transcriptase Gene (hTERT) Promoter Activity in Breast Cancer. *J. Biol. Chem.*, 2002; **277**: 20965-73.
24. Cox, D., Penney, K., Guo, S.E.H.Q., and Hunter, D.J. TGFB1 and TGFBR1 polymorphisms and breast cancer risk in the Nurses' Health Study. *BMC Cancer*, 2007;**7**:175.
25. Henderson, B.E., Feigelson, H.S. Hormonal carcinogenesis. *Carcinogenesis*, 2000; **21**:427-33.
26. Karadedou, C.T. Regulation of the FOXM1 transcription factor by the estrogen receptor á at the protein level, in breast cancer. *Hippokratia*, 2006;**10**:128–32.
27. Lin, Y., Huang, R., Chen, L., Li, S., Shi, Q., Jordan, C., *et al*. Identification of interleukin-8 as estrogen receptor-regulated factor involved in breast cancer invasion and angiogenesis by protein arrays. *Int. J. Cancer*, 2004;**109**:507-15.
28. Kim, Y.J., Cho, Y.E., Kim, Y.W., Kim, J.Y., Lee, S., Park, J.H. Suppression of putative tumour suppressor gene GLTSCR2 expression in human glioblastomas. *J. Pathol.*,2008; **216**: 218-24.
29. Kalt, I., Borodianskiy-Shteinberg, T., Schachor, A., Sarid, R. GLTSCR2/PICT-1, a Putative Tumor Suppressor Gene Product, Induces the Nucleolar Targeting of the Kaposi's Sarcoma-Associated Herpesvirus KS-Bcl-2 Protein. *J. Virol.*,2010; **84**: 2935-45.
30. Yu, J.L.F., Chen, H., Fu, J., Ray, S. Huang, S. Zheng, H., Ai, W. Kruppel-like factor 4 (KLF4) is required for maintenance of breast cancer stem cells and for cell migration and invasion. *Oncogene*, 2011; **30**: 2161-72
31. Yori, J.L., Johnson, E., Zhou, G., Jain, M.K., Keri, R.A. Krüppel-like Factor 4 Inhibits Epithelial-to-Mesenchymal Transition through Regulation of E-cadherin Gene Expression. *J. Biol. Chem.*,2010; **285**: 16854-63.
32. Davis-Dusenbery, B.N., Chan, M.C., Reno, K.E., Weisman, A.S., Layne, M.D., Lagna, G., et al. Down-regulation of Krüppel-like Factor-4 (KLF4) by MicroRNA-143/145 Is Critical for Modulation of Vascular Smooth Muscle Cell Phenotype by Transforming Growth Factor-â and Bone Morphogenetic Protein 4. *J. Biol. Chem.*, 2011; **286**: 28097-110.