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Emerging Role of Simvastatin in Targeting Triple Negative Breast Carcinoma Cells

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Keywords

• Simvastatin; Breast carcinoma; Proteomics

Abstract

Background: Recently, HMG-CoA reductase inhibitors are receiving larger importance in cancer therapeutics as they targets both metabolic and signal transduction platform. However, their functional role in targeting Triple negative breast cancer cells and its associated mechanism remains elusive.

Methods: In this study, growth inhibitory activity of simvastatin on MDA-MB-468 cells was assessed by MTT assay and its apoptotic potential by Nexin staining. Further, we employed label free quantitative proteomic profiling using mass tandem spectroscopy to explore the differentially expressed proteins associated with its anticancer activity.

Results: Treatment of MDA-MB-468 cells with increased concentrations of Simvastatin showed a remarkable growth inhibitory activity with the IC50 value of 9 µg/ml. Further, Nexin staining of the treated cells clearly indicates that, Simvastatin induces apoptosis in MDA-MD-468 cells. In Label free quantitative proteomic profiling of Simvastatin treated and untreated cells, 74 differentially expressed proteins were identified of which, 43 were up regulated and 31 were down regulated. Gene ontology and KEGG pathway enrichment analysis exposed 18 potential pathways associated with Simvastatin treatment. These identified pathways were shown to be related with focal adhesion, tumor progression, metastasis and metabolic effects in cancer cells. Among the down regulated proteins HSP90-alpha, Filamin-A, Alpha actinin-4, Vimentin and Phosphoglycerate kinase 1 was significantly down regulated.

Conclusion: These results imply that the application of Simvastatin is a possible new drug in the field of neoplasia to control growth and progression of breast carcinoma cells. Further our proteomic profiling reveals potential new drug targets for future drug development.

INTRODUCTION

Breast carcinoma accounts for very high lethality among women. Associated causes are metastasis, treatment resistance and lack of targeted therapeutics [1]. Although existing treatment modalities are effective in early stages, they have limited usefulness in treating triple negative ((ER-, PR-, HER2-) cases [2]. Hence developing a safe and effective therapeutic agent to combat TNBC is urgently needed and represents an intensive area in cancer research. Recently, 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG-CoA) inhibitors have received considerable attention in cancer therapeutics as they targets metabolic pathways associated with cancer progression and metastasis.

Earlier studies on several cell lines have shown that Statins targets the proliferation of neoplastic cells and induces cancer cell death [3-5]. Clinical studies using Statins demonstrated its influence the phenotype of breast tumors, suggesting a new potential strategy of breast cancer treatment [6,7]. In this study we have shown the growth inhibitory, apoptotic effect of Simvastatin on MDA-MB-468 triple negative breast cancer cell line. Further we employed label free quantitative proteomic profiling using mass tandem spectroscopy to study the underlying molecular mechanisms and associated pathways in its anticancer activity to find new molecular targets in drug treatment.

MATERIALS AND METHODS

Cell culture and reagents

MDA-MB-468 human breast cancer cells were obtained from Yenepoya Research Centre, Mangalore. The cells were tested for mycoplasma contamination (Mycoplasma Kit, LONZA, USA) and cultured with DMEM, supplemented with 10% FBS (Hyclone, UT, USA) and penicillin-streptomycin solution (Hyclone) and cultured at 37° C in 5% Co_2 incubator (Thermo scientific). For Label Free Quantitative (LFQ) proteomic analysis, Phosphate buffered saline ((PBS), Sodium Dodecyl Sulphate (SDS) and Urea were purchased from Ranchem, India. Ammonium bicarbonate (ABC), Acetonitrile (CAN), Dithiothreitol (DDT), Iodoacetamide (IAA) & Trifluoroacetic acid (TFA) were obtained from Sigma Aldrich, USA. Trypsin-Protease was purchased from Pierce (Thermo Scientific).

Activation of simvastatin

Simvastatin lactone (Sigma) was activated to its acidic form before the cell culture experiment. Briefly, 8 mg of Simvastatin was dissolved in 0.2 ml of 100% ethanol, with subsequent addition of 0.3 ml of 0.1 N NaOH. The solution was heated at 50°C for 2 h in a dry bath and neutralized with HCl to pH 7.2. The resulting solution was brought to a final volume (1 ml) with distilled water.

Cell proliferation assay

The cells were seeded in a 96 well plate at a density of 1×10^4 cells/ 100 µl/. After overnight incubation the cells were treated with different concentrations of Simvastatin for 48 hrs. During the last four hours, MTT reagent (5mg/ml) was added to each well. The production of MTT –formazan crystals was dissolved in 100 µl of DMSO and absorbance was measured at 560/ 620 nm. IC_{50} values were calculated using the GraphPad Prism Software, Version 5, USA.

Cell apoptosis study

The cells were seeded in 35-mm dishes at 2 x 10^5 cells/ dish and treated with different concentrations of Simvastatin or vehicle for 48 hrs. The cells were stained with Guava Nexin reagent and assay was carried out (Millipore, USA) according to the manufacturer's instructions. Apoptosis was detected using Guava EasyCyte Flow Cytometer (Merck Millpore,USA) and analyzed using FCS Express 6 plus research edition software.

Protein precipitation

For proteomic profiling, MDA-MB-468 cells were cultured and treated with 9 µg/ ml of Simvastatin for 24 hrs. Treated and untreated cells were suspended in Lysis buffer (4% SDS + 50 mM TEABC) and sonicated in ice, heat blocked at 90°C , centrifuged at 12,000 rpm, supernatant was collected and stored at -20°C till use. The protein concentration was measured by Bradford assay for normalization. 30 µg of protein from the control and treated lineages were subjected to acetone precipitation to remove salts and other interfering substances. The precipitated protein from each sample was dissolved in urea and the samples were processed immediately for trypsin digestion.

Reduction, alkylation, trypsin digestion and sample clean up

The protein pellets from each sample were dissolved by adding 10 µL of 6M urea and the volume was brought to 15 µL with HPLC grade water. Samples were then reduced by addition of 1.5 µL of 100 mM DTT and heated at 90°C for 10 mins. They were brought to room temperature and the reduced -SH groups were alkylated by adding 1.5 µL of 200 mM IAA and incubated in dark at RT for 15 min. 90 µL of ABC was added to the sample and proteins were digested by adding 1 µL of 1 mg/ml trypsin protease and incubated at 37 °C for 16 hours. The reaction was stopped by addition of 2 µL of concentrated TFA. The digested peptides were dissolved in 0.1% TFA, 5% ACN in water for MS-analysis.

Mass spectrometry analysis of proteins

HPLC-CHIP-MS: Agilent 1260 infinity HPLC-Chip/MS system is a microfluidic chip-based technology that incorporates peptide enrichment and separation and provides high-sensitive nano-spray. Charged peptides from HPLC-Chip system were directly infused into mass-spectrometer for detection. The following HPLC-Chip-MS conditions were used for acquiring the MS and MS/MS spectrum of the peptides. Chip ID: G4240-62030 Chip Name: High Performance Chip, 360 nanoliter enrichment column, 150 mm X 75 µm separation ,column Solvent A: 0.1% Formic Acid ,Solvent B:90% ACN / 10% (0.1% Formic Acid), Flow Rate: 0.3 µl / min Run Time:120 minutes, Sample Volume: 5 µl ,MS Scan Range: 275 to 1700 m/z ,MS Scan Rate: 8 spectra / sec, MS/MS Scan Rate: 3 spectra / sec Ion Polarity: Positive Ions Fragmentor Voltage:170 V, Skimmer Voltage:65 V ,Octopole RF Voltage:750V Gas ,Temperature: 250°C & Drying Gas: 5 L / min

Bio-informatics analysis of data: Protein identification was performed with the following criteria: (a) Trypsin digested peptides with 4 missed cleavages allowed, (b) peptide tolerance < 50 ppm, (c) > 2 unique peptides, (d) FDR < 5%. Fasta files for human proteins were downloaded from the uniprot database. For the analysis, proteins identified in at least 2 out of 3 replicates in each group were considered. Thus, a list of identified proteins was generated. Sum of Unique peptide intensity was used for semi-quantitative analysis. Ratio was calculated for proteins identified in both the treated and control groups. Ratio of >1.5 was considered as "up-regulated" and ratio < 0.5 was considered as down-regulated. The signaling pathway, Gene ontology and interaction network were analyzed using open source STRING (version 10) program (www.string-db.org).

Statistical analysis: The numerical data were represented as mean ± standard deviation. Student's t test or ANOVA were used to determine the difference between the groups using SPSS software version20 the significance level was set at $p < 0.05$ for all test.

RESULTS

Growth inhibitory and apoptotic effects of simvastatin

To study the growth inhibitory activity of Simvastatin on MDA-MB-468 triple negative breast carcinoma cells (TNBCs). The neoplastic cells were treated with various concentration of Simvastatin for period of 48 hrs. Treatment resulted in a significant growth inhibition with half inhibitory concentration of 9 µg/ml (Figure 1). All subsequent experiments were carried out using 9 µg/ml of Simvastatin. Further Annexin V and 7-AAD staining (Nexin Staining) for apoptosis revealed Simvastatin induces apoptosis in MDA-MB-468 cells (Figure 2A,B).

Overview of quantitative proteomics

Label free quantitative proteomics was performed in protein extracted from MDA-MD-468 cells with and without Simvastatin treatment to understand the cellular and molecular mechanism of its antitumor activity. Proteins were searched against human proteome database available from uniport. Filtering criteria of greater than 2 unique peptides with 5% FDR and a Q-value of zero was used. After data filtration 74 proteins showed significance difference in Simvastatin treated cells. Among the 74 differentially expressed proteins(DEPs) ,43 proteins were up regulated, intensity ratio > 1.5 (Table 1) and 31 proteins were

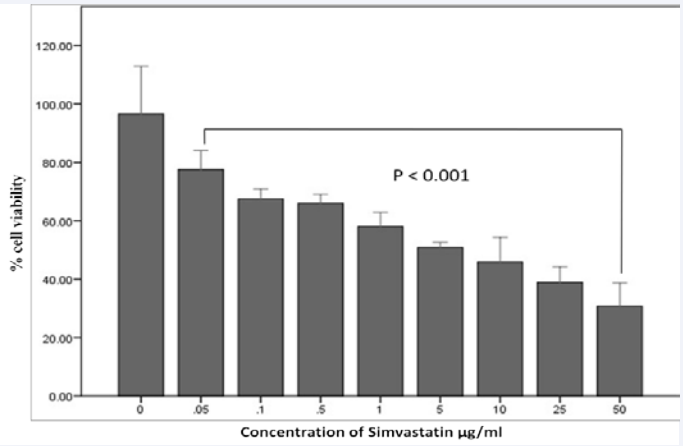

Figure 1 Simvastatin induces growth inhibition MDA-MB-468 seeded in 96-well plates were treated with different concentrations of simvastatin for 48 h. Cell proliferation was analyzed by measuring fluorescence at 620 nm after the addition MTT reagent. Data were represented as mean± standard deviation (n=3) P < 0.001 compared with the control.

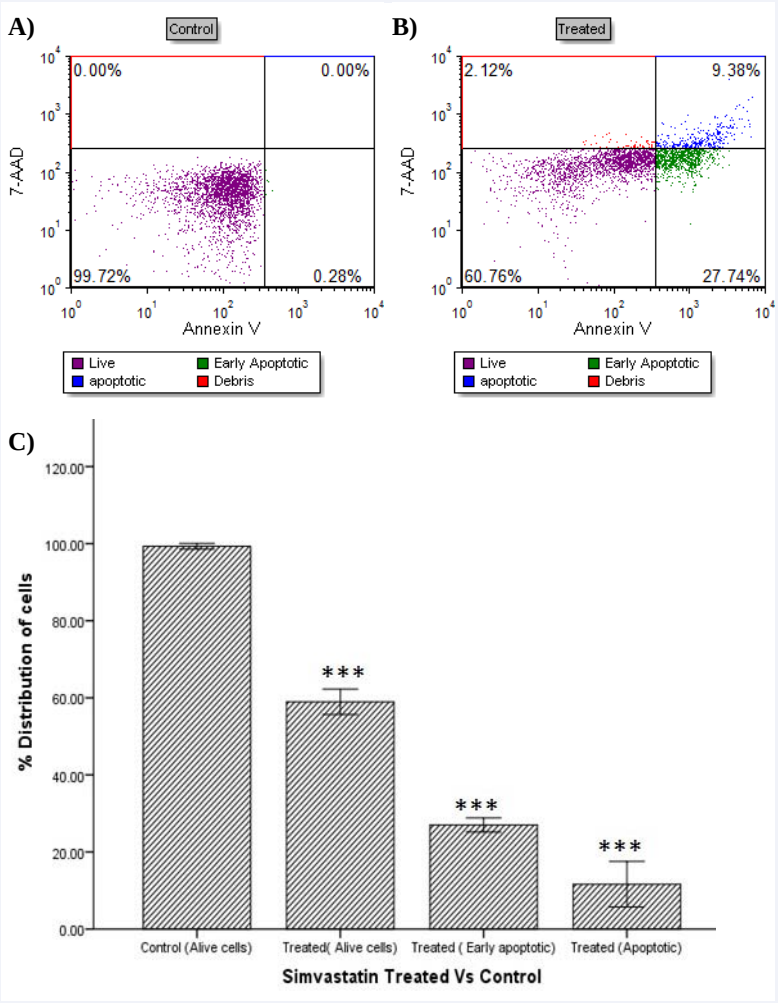

Figure 2 Simvastatin induces apoptosis in breast cancer cells (A) MDA-MB-468 cells seeded in 35-mm dishes were treated with simvastatin (9 µg/ml or vehicle and cultured for 48 h. The cells are stained directly with Guava Nexin Reagent , a pre-made cocktail containing Annexin-PE and 7-AAD in buffer, in a 200 µl final volume after a 20 –minutes incubation at room temperature, the samples are acquired on Guava system. (B) A bar graph represents the percentage distribution of cells in each related portion. Data were represented as mean ± standard deviation (n=3) P < 0.001 compared with the control.

down regulated proteins with intensity ratio <0.5 (Table 2).

The gene ontology analysis was performed using String database for all differentially regulated proteins for their molecular function (Figure 3A), biological process (Figure 3B) and cellular component (Figure 3C) approximately 39% were binding proteins and 20% were catalytic and enzyme regulators.

KEGG pathway enrichment analysis was also performed based on DEPs. a total of 18 pathways were identified $p < 0.05$ (Table 3) . This includes, Wnt signaling pathway (Catenin beta-1-P35222), Glycolysis/ Gluconeogenesis (Phosphoglycerate kinase 1-P00558), Pyruvate kinase PKM-P14618,Triosephosphate isomerase-P60174), PI3K-Akt signaling pathway (Heat shock protein HSP 90-alpha-P07900), Cell cycle (Protein kinase C inhibitor protein 1-P31946), Regulation of actin cytoskeleton (Profilin), Spliceosome (Poly(rC)-binding protein 1-Q15365), Adherens junction (Alpha-actinin-4-O43707) and Focal adhesion (Filamin-A-P21333)

DISCUSSION

Simvastatin, a potent HMG CoA reductase inhibitor is a well established lipid lowering drug in clinical settings. Its importance and clinical significance in cancer therapeutics are gaining significant importance. Many experimental, epidemiological and clinical studies imply that, Simvastatin possesses anticancer properties and increases the rate of survival among breast cancer patients. In addition, cancer cells are shown to exhibit elevated HMG-CoA reductase activity than the normal cells [8]. Further, mevalonate (MVA) pathway has been shown induce cellular proliferation and transformation in tumor cells [9,10]. The association of end products of MVA pathway with cancer progression and metastasis are well established [11]. More interestingly, it has been found that, Simvastatin is more sensitive and selective towards cancer cells than normal cells [12,13]. However their action in triple negative breast cancer cells and molecular mechanism remains elusive.

MDA-MD-468, a triple negative breast cancer cell line was used in this study. 48 hrs treatment of MDA-MB-468 cells with increased concentrations of Simvastatin showed a remarkable anticancer activity with the IC50 value of 9 µg/ml . Furthermore, Nexin (Annexin-V&7-AAD) staining of the treated cells clearly indicates that, Simvastatin induces apoptosis in MDA-MD-468 cells (Figure 2). Collectively, our results suggested that Simvastatin has the ability to inhibit cancer cell growth and induce apoptosis as being in agreement with previous studies [3-5].

Proteomics, specifically Label free quantitative proteomic profiling, is a unique method to investigate the drug action at its functional level. In this profiling of Simvastatin treated and untreated cells, 74 differentially expressed proteins were identified of which, 43 were upregulated and 31 were down regulated. Gene ontology and KEGG pathway enrichment analysis

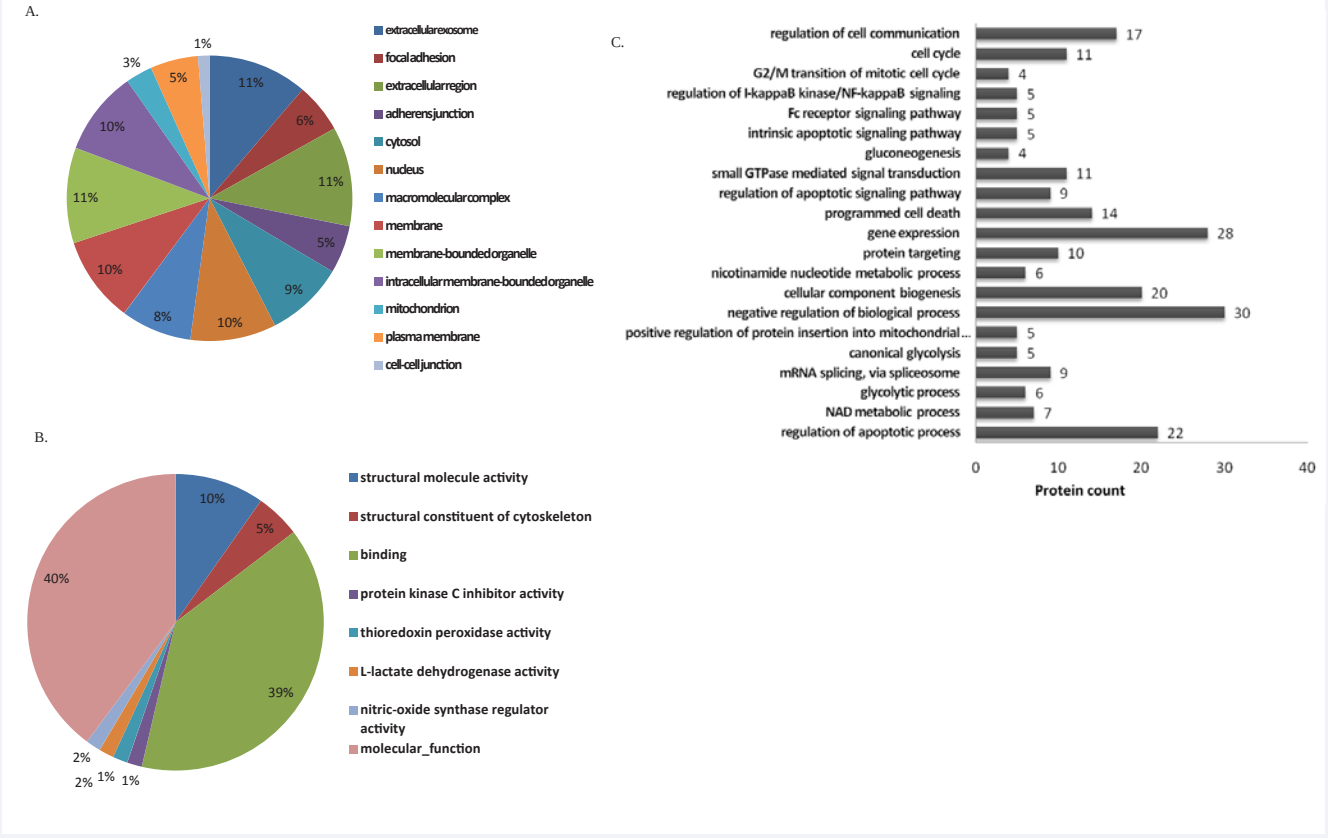

Figure 3 Categorization of all differentially expressed proteins by GO analysis (A) Cellular component (B) Molecular function (C) Biological process ($P<0.05$).

Table 1: Annotation of up-regulated proteins after Simvastatin treatment in MDA-MB-468 Cells.

Uniprota ID	Protein Sequence Coverage (%)	Protein names	Gene names	Number of Unique Peptides	Ratio/Fold change a*
P22314	7.9	Ubiquitin-like modifier-activating enzyme 1	UBA1	5	1.5
P13489	14	Ribonuclease inhibitor	RNH1	4	1.5
P08238	22.3	Heat shock protein HSP 90-beta (HSP 90)	HSP90AB1	11	1.5
Q15084	7.0	Protein disulfide-isomerase A6	PDIA6	5	1.5
P13639	7.9	Elongation factor 2 (EF-2)	EEF2	4	1.5
P62805	33.0	Histone H4	HIST1H4A	4	1.6
Q8IWP6	42.2	Tubulin beta chain		12	1.6
P68363	38.4	Tubulin alpha-1B chain	TUBA1B	10	1.6
P06733	16.1	Alpha-enolase)	ENO1	4	1.7
P13647	22.8	Keratin, type II cytoskeletal 5	KRT5	11	1.7
P06748	18.1	Nucleophosmin (NPM)	NPM1	4	1.7
Q53YD7	9.3	EEF1G protein (Eukaryotic translation elongation factor 1 gamma)	EEF1G	4	1.7
B0QZ18	7.4	Copine-1 (HCG38213, isoform CRA_b)	CPNE1 hCG_38213	4	1.7
P35232	14.3	Prohibitin	PHB	5	1.8
P11021	15.1	78 kDa glucose-regulated protein (GRP-78)	HSPA5 GRP78	4	1.8
P35579	8.6	Myosin-9	MYH9	10	1.8
V9HW80	11.8	Epididymis luminal protein 220	HEL-S-70	5	1.8
P08727	34	Keratin, type I cytoskeletal 19	KRT19	10	2.1
Q15366	33.75	Poly(rC)-binding protein 2 (Alpha-CP2)	PCBP2	4	2.1
A0A0C4DG17	27.3	40S ribosomal protein SA (37 kDa laminin receptor precursor) (37LRP) (37/67 kDa laminin receptor)	RPSA hCG_1997894	4	2.3
P05787	30.4	Keratin, type II cytoskeletal 8	KRT8	9	2.3
P05783	21.7	Keratin, type I cytoskeletal 18	KRT18	6	2.3
Q8N1C8	13.2	HSPA9 protein	HSPA9	4	2.4
Q9NX34	11.7	cDNA FLJ20465 fis, clone KAT06236		8	2.6
P07355	48.6	Annexin A2	ANXA2	13	2.6
G8JLB6	9.3	Heterogeneous nuclear ribonucleoprotein H	HNRNPH1	5	2.7
Q6IBN1	18.4	HNRPK protein	HNRPK HNRNPK hCG_1985922	5	2.7
P23246	8.6	Splicing factor, proline- and glutamine-rich (100 kDa DNA-pairing protein)	SFPQ PSF	4	2.7
Q00839	8.5	Heterogeneous nuclear ribonucleoprotein U (hnRNP U)	HNRNPU HNRPU	4	2.8
O60506	8.3	Heterogeneous nuclear ribonucleoprotein Q (hnRNP Q) (Glycine- and tyrosine-rich RNA-binding protein) (GRY-RBP) (NS1-associated protein 1) (Synaptotagmin-binding, cytoplasmic RNA-interacting protein)	SYNCRIP	4	2.8
Q09666	10.8	Neuroblast differentiation-associated protein AHNAK (Desmoyokin)	AHNAK	4	2.9
G9K388	30.9	YWHAE/FAM22A fusion protein (Fragment)	YWHAE/FAM22A fusion	4	3.1
A0A087WVQ6	3.5	Clathrin heavy chain	CLTC hCG_1818599	4	3.1
P07900	22.1	Heat shock protein HSP 90-alpha (Heat shock 86 kDa)	HSP90AA1	12	3.2
V9HWG3	21.1	Epididymis secretory protein Li 45	HEL-S-45	8	3.3
P31946	17.4	14-3-3 protein beta/alpha (Protein 1054) (Protein kinase C inhibitor protein 1)	YWHAB	4	3.4

P22626	21.5	Heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNP A2/B1)	HNRNPA2B1 HNRPA2B1	4	3.7
E7EX29	22.4	14-3-3 protein zeta/delta (Fragment)	YWHAZ	4	3.9
P02545	14.9	Prelamin-A/C [Cleaved into: Lamin-A/C (70 kDa lamin)	LMNA LMN1	7	3.9
P27348	13.4	14-3-3 protein theta	YWHAQ	7	4.4
P61981	11.5	14-3-3 protein gamma (Protein kinase C inhibitor protein 1) (KCIP-1)	YWHAG	5	5
Q15149	1.9	Plectin (PCN) (PLTN) (Hemidesmosomal protein 1) (HD1) (Plectin-1)	PLEC PLEC1	7	5.4
P11142	27.3	Heat shock cognate 71 kDa protein (Heat shock 70 kDa protein 8)	HSPA8 HSC70	10	5.4
a* matched ID ratio (Fold changes) of differentially expressed proteins in MDA-MB-468 cells (Simvastatin – treatment versus control)					

Table 2: Annotation of down-regulated proteins after Simvastatin treatment in MDA-MB-468 Cells.

Uniprot ID	Protein Sequence Coverage (%)	Protein names	Gene names	Number of Unique Peptides	Ratio/Fold change a*
P14618	23.9	Pyruvate kinase PKM	PKM	8	0.5
P04792	39.5	Heat shock protein beta-1 (HspB1	HSPB1	4	0.5
Q5U077	18.5	L-lactate dehydrogenase	HEL-S-281 LDHB hCG_24788	4	0.5
J3QQ67	18.4	60S ribosomal protein L18 (Fragment)	RPL18	4	0.5
I3L3D5	16.9	Profilin (Fragment)	PFN1	4	0.5
V9HW31	17.9	ATP synthase subunit beta (EC 3.6.3.14)	HEL-S-271	6	0.5
A8K486	26.6	Peptidyl-prolyl cis-trans isomerase (PPIase)		4	0.5
E9PK25	39.2	Cofilin-1	CFL1	4	0.5
Q6FI13	36.1	Histone H2A type 2-A (Histone H2A.2) (Histone H2A/o)	HIST2H2AA3 H2AFO	5	0.5
P62736	18.6	Actin, aortic smooth muscle (Alpha-actin-2) (Cell growth-inhibiting gene 46 protein)	ACTA2	5	0.5
P00338	8.1	L-lactate dehydrogenase A chain	LDHA	6	0.5
P60709	34.6	Actin, cytoplasmic 1 (Beta-actin)	ACTB	8	0.5
A0A024R321	10.6	Filamin B	FLNB	5	0.4
P32119	15.3	Peroxiredoxin-2	PRDX2	4	0.4
P10809	17.7	60 kDa heat shock protein, mitochondrial	HSPD1	6	0.4
P04406	28.3	Glyceraldehyde-3-phosphate dehydrogenase (GAPDH)	GAPDH	6	0.4
M0R0R2	20.8	40S ribosomal protein S5	RPS5	4	0.4
Q6FHZ0	19.6	Malate dehydrogenase	MDH2	4	0.4
Q59EJ3	6.9	Heat shock 70kDa protein 1A variant (Fragment)		4	0.4
Q15365	11.2	Poly(rC)-binding protein 1 (Alpha-CP1)	PCBP1	6	0.4
P08670	12.2	Vimentin	VIM	6	0.3
A0A0U1RRH7	52.1	Histone H2A		4	0.3
P35222	11.5	Catenin beta-1 (Beta-catenin)	CTNNB1	5	0.3
P07437	42.3	Tubulin beta chain	TUBB	12	0.3
P60174	30.4	Triosephosphate isomerase (TIM)	TPI1 TPI	6	0.2
Q06830	21.6	Peroxiredoxin-1	PRDX1	7	0.2
O43707	23.3	Alpha-actinin-4	ACTN4	16	0.2
P00558	11.7	Phosphoglycerate kinase 1	PGK1	6	0.2
V9HW26	9.5	ATP synthase subunit alpha	HEL-S-123m hCG_23783	4	0.2
P21333	6.9	Filamin-A (FLN-A)	FLNA	11	0.2
P14625	7.8	Endoplasmin (94 kDa glucose-regulated protein)	HSP90B1	4	0.2
a* matched ID ratio (Fold changes) of differentially expressed proteins in MDA-MB-468 cells (Simvastatin – treatment versus control)					

exposed 18 potential pathways associated with Simvastatin treatment (Table 3). These identified pathways were shown to be related with focal adhesion, cytoskeleton, metastasis and metabolic effects in cancer cells.

Among the down regulated proteins HSP90-alpha, Filamin-A, Alpha actinin-4, Vimentin and Phosphoglycerate kinase 1 were significantly down regulated.

Heat shock protein 90-alpha, a molecular chaperone plays an important role in stability, integrity and activation of proteins such as Erb2, Raf-1,Akt,HIF-α [14]. Higher expression of Hsp90-α is the common feature of all breast cancer [15,16]. In our study, Hsp90-α is significantly down regulated suggesting the potential role of Simvastain in combating the TNBCs. Hence, Hsp90-α can be proposed to be the potential target for drug development in cancer therapeutics.

The cytoskeletal Protein Filamin-A, was downregulated upon Simvastatin treatment in our study. Filamin-A have been shown to regulate cell division, metastasis, activation and signal transduction in neoplastic cells. Higher expressions were correlated with cancer development and progression in earlier studies [18,19]. In breast cancer, it has been shown that Filamin-A interacts with cyclin-D1 and initiate migration and invasion in breast cancer cells [20]. Further, higher expressions are shown to inhibit MMP-9 through MAPK/ERK pathways in tumor cell invasion [21]. Collectively, these findings suggest that Filamin-A as a new drug target in cancer prevention and treatment.

The expression level of Vimentin, a mesenchymal marker was significantly down regulated in Simvastatin treatment, vimentin clearly exhibits its association with apoptosis, multiple oncogeneic pathways, TNF signaling pathway and EMT .It is well known that targeted vimentin therapy reduces metastatic potency of the cancer cells [22,23]. These results demonstrated the potential use of Simvastatin in cancer therapeutics.

Other proteins involved in signaling pathways related to cancer which were significantly down regulated by Simvastatin include alpha actin-4 and Phosphoglycerate kinase 1. Increased expressions of alpha actinin-4 has been shown to be associated with signal transduction, nuclear translocation, gene expression and cellular movement in tumor cells [24,25]. It has been shown to be the versatile promoter of breast cancer tumroigenesis and appear to be future therapeutic target [24]. Phosphoglycerate kinase 1, a glycolytic enzyme that catalyzes the conversion of 1, 3-diphosphoglycerate to 3-phosphoglycerate in glycolysis have been shown to be associated with various malignancies [26,27]. In addition to the metabolic process it has been shown to regulate angiogenesis, DNA replication, repair and metastasis in various tumor [28]. In breast cancer higher expression are correlated with poor prognosis [29]. Overall, our study reveals that Simvastatin regulates multiple proteins involved in signaling pathways of cancer metastasis, progression, apoptosis, EMT and focal adhesion suggesting the a possible application of simvastatin in TNBCs.

Table 3: KEGG Pathway enrichment analysis of 74 Differentially expressed proteins in MDA-MB-468 cells treated with Simvastatin.

Pathway description	Observed protein count	False discovery rate p<0.05
Viral carcinogenesis	8	9.67E-06
Hippo signaling pathway	7	2.07E-05
Glycolysis / Gluconeogenesis	5	5.98E-05
PI3K-Akt signaling pathway	8	0.000364
Protein processing in endoplasmic reticulum	6	0.000393
Cell cycle	5	0.00105
Antigen processing and presentation	4	0.00137
Biosynthesis of amino acids	4	0.00158
Arrhythmogenic right ventricular cardiomyopathy (ARVC)	4	0.0017
Prostate cancer	4	0.00264
Estrogen signaling pathway	4	0.00349
Carbon metabolism	4	0.00467
Regulation of actin cytoskeleton	5	0.00707
Spliceosome	4	0.00872
Tight junction	4	0.00904
NOD-like receptor signaling pathway	3	0.0094
Adherens junction	3	0.0172
Focal adhesion	4	0.043

CONCLUSION

Our finding implies that the application of Simvastatin is a possible new drug in the field of neoplasia to control growth and progression of breast carcinoma cells. Further our proteomic profiling reveals potential new drug targets for future drug development.

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