p53 regulates the mevalonate pathway in human glioblastoma multiforme

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The mevalonate (MVA) pathway is an important metabolic pathway implicated in multiple aspects of tumorigenesis. In this study, we provided evidence that p53 induces the expression of a group of enzymes of the MVA pathway including 3′-hydroxy-3′-methylglutaryl-CoA reductase, MVA kinase, farnesyl diphosphate synthase and farnesyl diphosphate farnesyl transferase 1, in the human glioblastoma multiforme cell line, U343 cells, and in normal human astrocytes, NHAs. Genetic and pharmacologic perturbation of p53 directly influences the expression of these genes. Furthermore, p53 is recruited to the gene promoters in designated p53-responsive elements, thereby increasing their transcription. Such effect was abolished by site-directed mutagenesis in the p53-responsive element of promoter of the genes. These findings highlight another aspect of p53 functions unrelated to tumor suppression and suggest p53 as a novel regulator of the MVA pathway providing insight into the role of this pathway in cancer progression.

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The control of cellular metabolism is essential for a normal cell behavior, and the role that aberrant cellular metabolism has in cancer is becoming increasingly evident. During the course of tumorigenesis, changes in metabolism enable cells to survive and proliferate under adverse conditions that would directly arrest or kill a normal cell.1 Recent work has highlighted increased mevalonate (MVA) synthesis in malignant cells as consequence of increased levels and catalytic efficiency of 3′-hydroxy-3′-methylglutaryl-CoA reductase (HMGCR), the rate-limiting enzyme of cholesterol (CHO) biosynthesis that catalyzes the formation of MVA2 (Scheme 1). In addition, MVA is required for a number of cellular processes including DNA synthesis and proliferation.3 It is also the precursor of non-sterol isoprenoids endowed with a variety of functions including prenylation of growth-regulating proteins and oncoproteins.4 Elevated MVA synthesis has been reported in malignant breast cancer,5 lung,6 leukemia and lymphoma7 and hepatoma.8 Most recent results have shown that the administration of exogenous MVA to xenograft-bearing mice promoted tumor growth.9 Taken together, these results suggest that MVA pathway may have an important role in human malignancies. Indeed, recent transcriptional profiling demonstrated that CHO and lipid metabolism are linked to cellular transformation.10 However, it is not known whether the alterations of MVA pathway contribute to cancer etiology or whether its dysregulation occurs as a consequence of transformation. Given the importance of metabolic reprogramming in tumor development, it is not surprising that many oncogenes and tumor-suppressor genes have been shown to fine-tune these pathways.1,11 Their roles in carcinogenesis have traditionally been attributed to the ability to regulate the life and/or cell death.12 Anyway, evidence for an alternative concept, which the primary functions of activated oncogenes and inactivated tumor suppressors are to reprogram cellular metabolism, has continued to build over the past years. Among them, p53 seems to have an important role in regulating several aspects of cellular metabolism that are now being explored not only in cancer but in other diseases and in development.13 The p53 tumor-suppressor protein is a key factor in the cellular defense against carcinogenesis. It is a transcription factor that responds to numerous extrinsic and intrinsic challenges to the cell, including DNA damage, oncogene activation and hypoxia, to promote a variety of responses depending on the type, severity and persistence of the stress.13 An emerging striking observation is that by transcriptional activation and other means, p53 is able to contribute to the regulation of several pathways as oxidative phosphorylation, glutaminolysis, insulin sensitivity, nucleotide biosynthesis, mitochondrial integrity, fatty acid oxidation, antioxidant response, autophagy and mTOR signaling.14

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Abbreviations: MVA, mevalonate; HMGCR, 3′-hydroxy-3′-methylglutaryl-CoA reductase; MVK, mevalonate kinase; FDPS, farnesyl diphosphate synthase; FDF1, farnesyl diphosphate farnesyl transferase 1; RabGTTA, Rab geranylgeranyl transferase 1α; LDLR, LDL receptor; Eto, etoposide; PT-α, pifithrin-alpha; Nut, nutlin-3α; Lov, lovastatin; CHO, cholesterol

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Enzymes involved in Mevalonate pathway

- 3'-Hydroxy-3'-Methylglutaryl Coenzyme A Reductase
  - Mevalonate Kinase
  - Phosphomevalonate Kinase
  - Mevalonate-5-phosphate Decarboxylase
  - Isopentenyl Diphosphate Isomerase
  - Farnesyl Diphosphate Synthase
  - Farnesyl Diphosphate Farnesyl Transferase
  - Rab Geranylgeranyl Transferase 1α
  - Rab Geranylgeranyl Transferase 1β
  - CHOLESTEROL
  - LDL receptor

Scheme 1 Enzymes involved in MVA pathway

These evidences underscore the importance of the gene regulation achieved by p53. In this study, we found that p53 regulates the expression levels of some enzymes of the MVA metabolism in a human glioblastoma multiforme cell line and in their normal astrocytic counterpart. It recruited the genes promoters of these enzymes to activate their transcription. These results reveal a novel role of p53 in the systemic regulation of isoprenoid homeostasis.

Results

p53 induces the expression of MVA metabolism-related genes in human glioblastoma cells. High or deregulated activity of the MVA pathway has been demonstrated in several different tumors.15,16 In order to study the activity of this pathway in glioblastoma multiforme, we compared gene expression profiles of six genes relative to MVA pathway, including HMGCR, MVA kinase (MVK), farnesyl diphosphate synthase (FDPS), farnesyl diphosphate farnesyl transferase 1 (FDFT1), Rab geranylgeranyl transferase 1α (RabGGTA) and LDL receptor (LDLR) (Scheme 1) in U343 cells expressing wild-type p53 and U251 cells with mutant p53 (R273H), carrying a mutation abolishing the DNA-binding activity.17 Normal human astrocytes, NHAs, were used in comparison. Quantitative real-time PCR (qRT-PCR) measurements were conducted for mRNA levels of aforementioned genes (Supplementary Table 1). As shown in Figure 1a, the obtained data revealed that they were significantly upregulated at basal level in U343 cells expressing wild-type p53 and downregulated in U251 cells with mutant p53 (R273H), compared with NHA, a NHA cell line. In this context, the mRNA levels of CDKN1A (p21waf) and GADD45, known p53 target genes,18 paralleled with p53 activity. In order to verify if p53 activation induced the expression of the genes involved in the MVA pathway, we performed qRT-PCR measurement of the mentioned genes in U343 cells treated with Nutlin 3a (Nut), which is known to stabilize and activate p53.19 As shown in Figure 1c, we observed that the expression levels of MVA pathway genes were induced in Nutlin 3a-treated cells compared with untreated cells. Furthermore, we evaluated by western blot analysis (Figure 1b) the protein levels of the enzymes of the MVA pathway and observed that they were overexpressed in NHA cells and U343 cells untreated and treated with Nutlin 3a in comparison with U251 cells, thus confirming proper p53 activity in our experimental setup (Figure 1c). In parallel, in order to demonstrate the transcriptional regulation of these genes by p53, we transfected U343 cells with either an empty vector (mock) or a vector encoding the mutant p53 (R273H) (Figure 2a). The ectopic expression of a dominant-negative mutant p53 (R273H) in U343 cells significantly attenuated the mRNA levels of the six MVA pathway genes analyzed (Figure 2a). The transfection efficiency has been confirmed by western blot analysis (Figure 2a). To support the role of p53 in transcriptional regulation of the enzymes of MVA pathway, we knocked down p53 using a specific p53-siRNA. We observed that the reduction of p53, as shown in western blot in Figure 2f, turned down both the expression and protein levels of the tested enzymes (Figures 2d and f). Then, to corroborate our findings, we examined the expression of all these genes following ectopic expression of wild-type p53 in HEK293 cells. As shown in Figure 2c, the overexpression of wild-type p53 in HEK293 cells, significantly induced the mRNA levels of all genes compared with cells transfected with empty vector (mock), instead the overexpression of mutant p53 alone reduced the expression of analyzed genes. The western blot in Figure 2e shows the transfection efficiency. The role of p53 on these genes was confirmed, at least in part, in U251 cells, after transfection with a vector bearing wild-type p53 (Figure 2b). In order to further generalize our findings, we tested the ability of p53 to activate the MVA metabolism-related genes in human adenocarcinoma breast cancer cell lines MCF7 with wild-type p53 and MDA MB 231 with p53 mutant (R280T)17 and in human adenocarcinoma colon cancer cell lines HCT116 with wild-type p53 and SW620 with p53 mutant (R273H).17 In accordance with human glioblastoma cells used in this study, levels of six genes were increased in cell lines with wild-type p53 compared with cell lines with mutant p53 (Supplementary Data,Supplementary Figure S1).

p53 is recruited to p53-responsive elements in the promoters of MVA pathway-related genes thereby inducing their transcription. p53 induces transcription of its primary target genes by a direct binding of PuPuPuC(AT) (TA)GPypPyPy sequence presented in p53-responsive element (p53RE) of DNA regulatory elements (promoters and enhancers).20 Using Genomatix MatInspector,21 we searched for putative p53REs in the promoters of the genes relative to MVA pathway. These genomic regions consisted of 3-kb upstream and 2-kb downstream relative to transcription start sites, whereas for FDFT1, MatInspector software identified three sites located into intron in gene-coding sequence. Each gene contained a putative p53RE in its promoter (Supplementary Table 2). In order to confirm that p53 binding to these sites, we performed chromatin immunoprecipitation (ChIP) in U343 cells, U251 cells transfected with an empty vector (mock) or a vector encoding the wild-type p53 and in NHA cells, treated or not with etoposide
(Eto) at 25 μM for 24 h. Then, we analyzed the amount of DNA precipitated with an antibody against p53 or a control antibody (IgG), using specific primers for the putative binding site located in promoters in different areas relative to transcriptional start site of each gene (Table 3). Genomic regions of HMGCR, MVK, FDPS and FDFT1 were strongly enriched in the p53-immunoprecipitated chromatin, indicating that p53 is recruited to the consensus sequence of these genes (Figures 3a and b), whereas those of RabGGTA and LDLR were only enriched in the NHAs (Figures 3c and d). We validated the ChIP findings by analyzing the p53-binding sites at the canonical p53 target CDKN1A (p21waf). In order to distinguish between the endogenous and transfected p53, we performed a CHIP assay in U251 cells transfected with FLAG-p53 expression vector or with an equivalent amount of empty vector, as shown in Figure 3f. Then, we analyzed the amount of DNA immunoprecipitated with anti-FLAG antibodies or a control antibody (IgG), using specific primers for the putative binding site located in promoters in different areas relative to the transcriptional start site of each gene (Table 3). Genomic regions of HMGCR, MVK, FDPS and FDFT1 were strongly enriched in the anti-FLAG-immunoprecipitated chromatin, indicating that the transfected p53 was recruited to the consensus sequence of these genes (Figures 3e and g). Next, we tested the functionality of the identified p53REs. To this end, we cloned the DNA regions containing the putative p53RE of HMGCR, MVK, FDPS, FDFT1, RabGGTA and LDLR into a luciferase reporter plasmid (Scheme 2; Table 2) and carried out a series of promoter activity experiments in U343 cells transfected with luciferase reporter plasmids bearing promoter and intronic regions of enzymes alone (Figure 4a) or in co-transfection with mutant p53 (R273H) (Figure 4b) and in U251 cells co-transfected with wild-type p53 compared with cells co-transfected with mock (Figure 4c) or reporter plasmids alone (Figure 4d). Luciferase expression was significantly enhanced in a p53-dependent manner in the constructs harboring the promoters of HMGCR, MVK, FDPS, FDFT1 (Figures 4a and d), whereas no luciferase activity in cell lines transfected with plasmids bearing RabGGTA and LDLR promoters was observed (data not shown). Moreover, in U343 cells, the same promoters showed reduced luciferase activity when co-transfected with the mutant p53 (Figure 4b). In order to confirm that the p53REs in the promoters are responsible for the elevation in luciferase activity, we mutated the genomic regions in the p53-binding sites. We carried out two types of mutations: (1) deletion of the region bearing the p53RE (Figure 4; Table 3) and (2) single mutations of core nucleotides in the p53RE within the cloned fragment (Scheme 2; Table 4). Very interestingly, all the mutants significantly reduced the p53-dependent induction of luciferase activity (Figures 4a and c). Taken together, these findings suggest that p53 could bind to the specific
region of promoters of MVA metabolism-related genes inducing their transcription.

**p53 is recruited to the promoters of MVA pathway enzymes independently from SREBP proteins.** The physiologic regulation of MVA pathway enzymes requires the sterol regulatory element-binding proteins. Recently, Freed-Pastor et al. have described that the mutant p53 is recruited at the promoters of sterol genes by SREBP proteins to upregulate the expression of the sterol biosynthesis enzymes in human breast cancer cells. In order to test the physiologic relevance of the enzymes of MVA pathway (f). A representative actin was used for all samples as loading control. (e) Western blot analyses shows the overexpression of p53 after transfection in HEK293 cell line in the several experimental conditions. ***P ≤ 0.001

**SREBP1 and SREBP2.** qRT-PCR measurements after depletion of SREBP1 and SREBP2 in U343 cells (as shown in Figure 5d) substantially decreased the expression levels of the enzymes but knockdown of p53 profoundly attenuated the enzymes expression (Figure 5a). Moreover, we carried out a quantitative ChIP analysis and observed that the ChIP signal was consistently reduced upon depletion of p53 in comparison with depletion of SREBP1 and SREBP2 (Figure 5b). Next, the activity of promoters of the enzymes revealed that the depletion of SREBP1 and SREBP2 slightly reduced the activation of a luciferase reporter under the control of cloned promoters of the enzymes in comparison with the depletion of p53 (Figure 5c). Next, to evaluate the effects of knockdown of SREBP1 and SREBP2 on the transcription of tested enzymes, we transiently co-expressed FLAG-p53 with specific siRNA of SREBP1 and SREBP2 in U251 cells (as shown in Figure 5d). Western blot showed p53 protein levels following transfections with the plasmid constructs in U343 cells (ctr = untransfected cells; mock = cells transfected with empty vector; U343+p53(R273H) = cells transfected with mutant p53). (b) U251 cells transfected with empty vector control or with vector containing wild-type p53 (pCMV/p53). Western blot shows p53 protein levels following transfection. (c) HEK293 cells transfected with control empty vector (mock), or with pCMV/p53 alone, co-transfected with construct containing with mutant p53 or transfected with empty vector (mock) or with construct containing with mutant p53. (d) Knockdown of wt p53 from U343 cell line: cells transfected with siRNA p53 downregulated the expression and the protein levels of enzymes of MVA pathway. A representative actin was used for all samples as loading control. (e) Western blot analyses shows the overexpression of p53 after transfection in HEK293 cell line in the several experimental conditions. ***P ≤ 0.001

**Figure 2** Analysis of mRNA levels of enzymes related to MVA pathway upon genetic perturbation of p53. qRT-PCR analysis of MVA pathway enzymes in (a) U343 cells untransfected and transfected with empty vector control (mock) or with mutant (R273H) p53. Data were analyzed with a two-tailed t-test. Western blot showed p53 protein levels following transfections with the plasmid constructs in U343 cells (ctr = untransfected cells; mock = cells transfected with empty vector; U343+p53(R273H) = cells transfected with mutant p53). (b) U251 cells transfected with empty vector control or with vector containing wild-type p53 (pCMV/p53). Western blot shows p53 protein levels following transfections. (c) HEK293 cells transfected with control empty vector (mock), or with pCMV/p53 alone, co-transfected with construct containing with mutant p53 or transfected with empty vector (mock) or with vector containing with mutant p53. Western blot shows the transfection efficiency. Data are mean ± S.D. of three independent experiments. One-way ANOVA analysis indicated a significant difference in genes expression (***P ≤ 0.001; *P ≤ 0.05). (d) Knockdown of wt p53 from U343 cell line: cells transfected with siRNA p53 downregulated the expression and the protein levels of enzymes of MVA pathway. A representative actin was used for all samples as loading control. (e) Western blot analyses shows the overexpression of p53 after transfection in HEK293 cell line in the several experimental conditions. ***P ≤ 0.001

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shown in Figure 6b) and carried out a ChIP assay (Figure 6a).

The chromatin region was immunoprecipitated with anti-FLAG, thus indicating the p53 binding to promoter regions of enzymes after depletion of SREBP1 and SREBP2 (Figure 6a). Luciferase assay of the enzymes revealed that the knockdown of SREBP1 and SREBP2 causes a weak and irrelevant reduction of the activity of promoter regions of enzymes (Figure 6c).

p53 modulates CHO biosynthesis in glioblastoma cells.

As p53 regulates the expression of some enzymes of MVA pathway, including HMGCR, the key regulatory enzyme of isoprenoids synthesis, we measured the synthesis of CHO product of MVA pathway in the human glioblastoma multiforme U343 and U251 cell lines and in NHA. In basal condition, we observed an increase in the incorporation of [14C]-acetate in CHO in U343 cells compared with U251 cells.
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and their normal astrocytic counterpart (Figure 7). In any case, the presence of Lovastatin (Lov; 10 μM for 24 h; a competitive inhibitor of HMGCR activity, inhibited the CHO synthesis in U343, NHA and U251 cell lines; moreover, a transcriptional inhibitor of p53, cyclic pifithrin-alpha (PT-α; 30 μM for 24 h) decreased the incorporation of [14C]-acetate in CHO product in all cell lines (Figure 7).

Discussion

Malignant gliomas are highly dependent on the MVA pathway for the synthesis of CHO and isoprenoid compounds compared with their normal counterpart. This pathway is highly regulated in untransformed cells, and loss of any one of these regulatory mechanisms may contribute to dysregulation of this critical pathway and ultimately drive tumorigenesis. In this study, we analyzed the expression profiles of some enzymes relative to this pathway in two different cell lines of glioblastoma multiforme, U343 cells expressing a wild-type p53, inhibited the [14C]-acetate incorporation in CHO in U343 and U251 cells suggesting a correlation between p53 and CHO pathway. The results shown here suggest that in U343 cells, p53 could be the major regulator of the MVA pathway allowing the elevation of CHO metabolism essential for cellular survival. The hypothesis currently under investigation is that the upregulation of CHO metabolism could then drive tumorigenesis. Recent evidence have described that, although p53 is a tumor suppressor, some of its downstream targets do not have a tumor-suppressive function. The research of the pro-survival function of p53 is essential for cancer therapy because the identification of novel treatments targeting the pro-survival function of p53 could direct the cancer cells toward
apoptosis, thus allowing the acquisition of optimal efficacy for chemotherapy. We observed that U251 cells bearing mutant p53, although unable to upregulate the sterol biosynthesis genes, showed a high rate of endogenous CHO synthesis allowing the elevated rate of cell proliferation, to understand this mechanisms are needed for further studies. We believe that the reduction of sterol biosynthesis genes levels and, as consequence, a reduced amount of intracellular CHO make this cell type particularly sensitive to the inhibition of this pathway.31 Indeed, the treatment with Lov reduced considerably the amount of free CHO (Figure 7). In addition, most of glioma cells with wild-type p53 exhibit a high resistance to cytotoxic treatments used in clinical practice compared with glioma cells with transcriptionally inactive mutant p53.32 It appears that p53 activities associated with DNA repair contribute to the overall survival potential and drug resistance. Supporting the notion, there is evidence that chemoresistance mediated by p53 in glioma relies at least in part on the ability of p53 to activate the transcription of DNA repair genes,33 and of genes whose products directly contribute to drug resistance of glioma to temozolomide such as MGMT (O6-methylguanine-DNA methyltransferase), an enzyme that removes methyl and chloroethyl groups from the O6-position of guanine.34 Furthermore, the effects of p53 on drug resistance and the survival potential seen in different types of cancer cells, could be due to the activation of CHO pathway that, in order to support cell survival, may modulate resistance to a variety of anticancer drugs, within a functional phenotype termed multidrug resistance (MDR). The plasma membranes of MDR+ tumor cells are rich in fact, in CHO that assists the activity of Pglycoprotein (Pgp), an integral membrane transporter chemotherapy drugs. Another hallmark of MDR+ tumor cells is the increased prenylation and activity of GTP-binding proteins, which are also dependent on the rate of the MVA pathway activity.35 In summary, our results show that p53 is an essential transcription factor for the regulation of MVA pathway, and further studies will be necessary to determine the mechanism by which p53 regulates the MVA pathway enzymes. A better understanding of how p53 coordinates metabolic adaptation will facilitate the identification of novel therapeutic targets and will also illuminate the wider role of p53 in human biology.

Materials and Methods

Cell cultures and reagents. NHAs are normal human cells derived from human brain tissue and were cultured in AGM BulletKit medium (Lonza, Portsmouth, NH, USA), these cells were used as normal control system.
Glioblastoma multiforme cell lines U343 and U251 cells (CLS, Berlin, Germany) were maintained in EMEM, supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, 0.1 mM non-essential amino acids, 1.0 mM sodium-pyruvate. Human embryonic kidney 293 cells (HEK293) were maintained in DMEM supplemented with 10% FBS. All cell lines were cultured at 37 °C in 5% CO₂ controlled atmosphere. Reagents Eto, PT-α and Nut were purchased from Sigma-Aldrich S.r.l. (Milan, Italy). Cells were treated with Eto 25 μM for 24 h, PT-α was used at a concentration of 30 μM for 24 h, whereas Nut was administered at 10 μM for 24 h. Lov, a gift from Dr. AW Alberts of the Merck, Sharp and Dohme Institute (Kenilworth, NJ, USA), was used at 10 μM for 24 h of treatment. siRNA targeting SREBP1 (h) (sc36557) was purchased from Santa Cruz Biotechnology (Dallas, TX, USA). siRNA p53 (106141), SREBP2 (s27) and Lipofectamine RNAiMAX reagent were from Invitrogen (Carlsbad, CA, USA).

**Immunoblotting.** Cells were harvested after 24-h treatment, washed twice with phosphate-buffered saline (PBS) and resuspended in lysis buffer with protease inhibitors (Hepes 50 mM, NaCl 150 mM, EDTA 50 mM, NaF 100 mM, Na ortovanadate 2 mM, glycerol, Na4P2O7 10 mM, 10% Triton pH 7.5, all compounds were from Sigma, Milan, Italy). Protein concentration was determined by Bio-Rad Protein Assay (Bio-Rad, Carlsbad, CA, USA). 50 μg of proteins were resuspended in Laemmli sample buffer and loaded onto a SDS-PAGE. Samples were resolved under constant voltage (40 mA) and transferred onto nitrocellulose blot (100 V).

**Figure 5** (a) qRT-PCR of enzymes related to MVA pathway, (b) ChIP assays and (c) luciferase reporter analysis of U343 cells after knockdown of SREBP1, SREBP2 and p53. U343 cells were treated with a control siRNA or siRNA directed against SREBP1, SREBP2 and p53. (d) Immunoblot shows knockdown of SREBP1 and SREBP2 (see also Figure 3f for the depletion of p53). Actin was made as control of loading. Data represent mean ± S.D. of three independent experiments and statistical analysis was performed with one-way ANOVA, *P ≤ 0.05; **P ≤ 0.01.

**Figure 6** (a) ChIP assays and (c) Luciferase reporter analysis of U251 cells after knockdown of SREBP1 and SREBP2. U251 cells were co-transfected with FLAG-p53 and control siRNA or siRNA directed against SREBP1 and SREBP2. (b) Immunoblot shows knockdown of SREBP1 and SREBP2. Actin was made as control of loading. Data represent mean ± S.D. of three independent experiments and statistical analysis was performed with one-way ANOVA, *P ≤ 0.05; **P ≤ 0.01.
Blots were blocked in PBS containing 0.1% Tween 20 and 5% non-fat milk for 1 h at room temperature. Filters were probed overnight with primary specific antibodies at 4 °C: mouse anti-p53 (sc-126), rabbit anti-p21waf (sc-756), rabbit anti-HMGCR (sc-271595), rabbit anti-MVK (sc-365513), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDPT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), anti-mouse SREBP1 (sc-365513) and anti-SREBP2 (sc-5603) were from Santa Cruz Biotechnology; rabbit anti-FDFT1 (sc-99145), rabbit anti-RabGGTA (sc-133617), mouse anti-LDL (sc-18823), ant
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