Proteomic analysis and comparison of intra- and extracranial cerebral atherosclerosis responses to hyperlipidemia in rabbits

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Abstract. The present study aimed to investigate protein expression levels of intra- and extracranial atherosclerosis in rabbits following administration of a high-fat diet. Rabbits were randomly divided into control (group A; n=9) and high-fat diet (group B; n=9) groups. At week 12, tissues were sectioned from the common carotid artery (CCA) and middle cerebral artery (MCA). Pathological analysis was performed. Differential protein expression levels were examined by 2-D gel electrophoresis (2-DE) and mass spectrometry (MS) analysis and validated by western blotting. Serum lipid levels, the intima-media thickness (IMT) and degree of atherosclerosis of the CCA and MCA were increased at week 12 in the high-fat diet group compared with rabbits that received a normal diet. 2-DE and MS analysis of the protein extracted from CCA and MCA detected >439 different proteins; the expression of 25 proteins was altered, and 8 proteins [albumin A chain, tropomyosin α-1 chain (TPM1), heat shock protein 70 (HSP70), α-smooth muscle actin, β-galactose binding agglutinin, TPM4 isoform 2, cell keratin 9, single octylic acid glyceride β-2) demonstrated significant alterations in expression levels. Due to limited antibody sources, only three differentially expressed proteins (TPM1, HSP70 and α-smooth muscle actin) were examined by western blotting. The results of our previous study demonstrated that hyperlipidemia affected the IMT of intracranial and extracranial cerebral arteries. In the present study, protein expression levels of TPM1 and α-smooth muscle actin from extracranial cerebral arteries were significantly increased compared with intracranial cerebral arteries; however, protein expression levels of HSP70 from intracranial cerebral arteries was increased compared with extracranial cerebral arteries. The differences may be closely associated with cell proliferation and metastasis, and oxidoreduction, in intra- and extracranial cerebral atherosclerosis. HSP70 may have protective properties against atherosclerosis via underlying anti-inflammatory mechanisms, furthermore, differential protein expression levels (TPM1, HSP70 and α-smooth muscle actin) between intra- and extracranial cerebral arteries may facilitate the identification of novel biological markers for the diagnosis and treatment of cerebral arteriosclerosis.

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Introduction

Cerebral atherosclerosis, the primary cause of ischemic stroke, is divided into extracranial atherosclerosis (ECAS) and intracranial atherosclerosis (ICAS) (1-3). The underlying molecular mechanisms of ICAS and ECAS have been extensively studied; however, remain to be fully elucidated. Cerebral atherosclerosis is influenced by various risk factors, including hypertension, hyperlipidemia, obesity, smoking and diabetes. Previous studies have reported that blood cholesterol levels and associated lipoprotein levels are associated with the risk of coronary artery disease; however, this is weakly associated in ECAS and ICAS, suggesting potential variations in underlying mechanisms or susceptibility (2,4,5). The role of hyperlipidemia in ICAS and ECAS remains unclear due to controversial previously reported findings. A previous study conducted proteomics analyses of the common carotid artery by obtaining human carotid atherosclerotic plaques, and demonstrated certain proteins are in low-abundance, including heat shock protein 27 (HSP27) isoforms, aldehyde dehydrogenase, moesin, protein kinase C δ-binding protein and inter-α-trypsin inhibitor family heavy chain-related protein are correlated with biological alterations associated with atherosclerosis (6). In our previous study, HSP70 expression levels were demonstrated to vary between ICAS and ECAS; however, the effect of other protein expressions on ICAS and ECAS remains unclear (7). Investigation into the impact of hyperlipidemia on ICAS and ECAS have yielded controversial results, and it is unclear whether any specific target proteins are involved in the discrepancies between ICAS and ECAS. A more global analysis of the activities of all relevant molecules is required, which may provide a complete view of their interactions. Proteomic analysis has previously been performed to identify novel therapeutic targets for use in the treatment

and prevention of atherosclerosis (8). The present study aimed to compare proteomic and biomarker profiles associated with cerebral ICAS and ECAS. The identification of protein biomarkers that underlie cerebral atherosclerosis may provide valuable diagnostic indicators and therapeutic targets for the treatment of the disease.

Materials and methods

Animals. Male New Zealand White rabbits (n=18; weight, 2.0-2.5 kg) were provided by the Animal Laboratory of Tongji University (Shanghai, China). They were fed regular rabbit feed for one week prior to the experiment and were kept in a temperature-controlled environment (20±1˚C; humidity, 55±5%) under a 12-h light/dark cycle in an air-conditioned room. Rabbits were randomly divided into two groups: Control (A; n=9) and high-fat (B; n=9). Rabbits in group A continued to receive regular rabbit feed and group B were fed a high-fat diet (2% cholesterol, 6% peanut oil and 92% basic feed) for 12 weeks based on the methods of previous studies (6,9,10). Rabbits in groups A and B were fed restricted diets with an equal amount of food (150-200 g per day) and free access to water. The present study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The animal use protocol was reviewed and approved by the Institutional Animal Care and Use Committee of Tongji University School of Medicine (Shanghai, China).

Lipid profile and histopathological examination. Circulation blood samples were collected from the rabbits at weeks 0, 4, 8 and 12 on the different diets. The rabbits in groups A and B were sacrificed at weeks 0, 4, 8 and 12. For groups A and B, 3 rabbits were sacrificed at each time point (4,8 and 12 weeks). Artery segments [including the common carotid artery (CCA) and middle cerebral artery (MCA)] were dissected and fixed in 4% paraformaldehyde for histomorphometry observation. Pathological analysis was performed by hematoxylin and eosin (H&E) staining, as described previously (7). Images were observed under a light microscope, following which intima-media thickness (IMT) was measured using Image Pro Plus software version 6.0 (Media Cybernetics, Inc., Rockville, MD, USA). The tissues were stored at -80˚C for 2-D gel electrophoresis (2-DE) and proteins expression levels were analyzed by western blotting.

2-DE and silver staining. CCA and MCA tissues from groups A and B were ground to a powder using liquid nitrogen, detailed process as follows: Homogenized in ice-cold homogenizing buffer [9 M urea; 4% w/v CHAPS; 1% w/v dithiothreitol (DTT); 0.5% chilled acetone (CA) and a cocktail of protease inhibitors]. The homogenate was centrifuged at 3,500 x g for 30 min at 6˚C and the precipitation was collected and suspended in precooling acetone containing 0.2% DTT, and frozen at -20˚C for 1 h. Precipitated proteins were centrifuged for 30 min at 5,000 x g at 6˚C, which was followed by two additions of CA. The pellets were air dried at room temperature and dissolved overnight at 4˚C in lysis buffer containing 7 M urea, 2 M thiourea, 4% w/v CHAPS, 1% w/v DTT, 0.5% ampholyte and a cocktail of protease inhibitors. Tissue proteins were fractionated as described previously (11-13) and protein concentrations were determined using the Bradford assay according to methods described previously (14), and subsequently stored at -80˚C for isoelectric focusing (IEF).

2-DE and silver staining were performed. Protein sample (200 μg) was mixed with fresh rehydration buffer to a total of 450 μl, following which IEF was performed using an Immobiline DryStrip (IDS) gel (GE Healthcare Life Sciences, Chalfont, UK; length, 24 cm; pH 3-10; non-linear gradient) at room temperature for 10 min. Following a two-step equilibration, proteins on the IPG strips were separated by SDS-PAGE using the Ettan-DALTsix Electrophoresis system (GE Healthcare Life Sciences) at 15˚C and 200 V for 6-8 h. The IDS gel was visualized by silver staining according to the protocol of Shevchenko *et al* (15). The stained gel was scanned using an ImageScanner system (GE Healthcare Bio-Sciences, Pittsburgh, PA, USA) at a resolution of 300 dots per inch. All gel images were processed by three steps using PDQuest 2-D Analysis software version 8.0 (Bio-Rad Laboratories, Inc., Hercules, CA, USA) as follows: Spot detection, volumetric quantification and matching. Differences in protein content between high-fat diet and control groups were analyzed by Student's t-test (n=3) and calculated as fold ratio. Fold-change ≥1.5 or ≤0.67 was used to select differentially expressed protein spots.

Mass spectrometry (MS) analysis. The extracted protein samples were re-suspended with 5 μl 0.1% trifluoroacetic acid (TFA) followed by mixing in a matrix consisting of a saturated solution of α-cyano-4-hydroxy-trans-cinnamic acid in 50% acetonitrile and 0.1% TFA at a 1:1 ratio. Mixture (1 μl) was spotted onto a stainless-steel sample target plate. Peptide MS and MS/MS were performed on an AB SCIEX 5800 time-of-flight (TOF)/TOF™ system mass spectrometer (Applied Biosystems; Thermo Fisher Scientific, Inc., Waltham, MA, USA). MS and MS/MS datasets were integrated and processed using GPS Explorer™ software version 3.6 (Applied Biosystems; Thermo Fisher Scientific, Inc.) with default parameters. Proteins were successfully identified based on 95% or higher confidence interval of their scores in the Mascot v2.1 search engine (Matrix Science, Ltd., London, UK) using the Mascot and NCBI protein databases. Peptide mixtures were separated on an UltiMate™ 3000 Nano Liquid Chromatography system (Dionex; Thermo Fisher Scientific, Inc.) as described by Moller *et al* (11).

Western blotting. Proteins were extracted as described above and protein lysates (20 μg per lane) were separated by one-dimensional 10% SDS-PAGE and were transferred onto nitrocellulose membranes. Membranes were blocked with PBS containing 5% nonfat dry milk at 4˚C overnight and were subsequently incubated with the following primary antibodies: Polyclonal mouse anti-α-smooth actin (cat. no. ab7817, 1:300), mouse anti-HSP70 (cat. no. ab2787, 1:100), rabbit anti-tropomyosin α-1 chain (cat. no. ab55915, TPM1; 1:500) (all from Abcam, Cambridge, UK) and mouse GAPDH (cat. no. sc-365062; 1:1,000; Santa Cruz Biotechnology, Inc., Dallas, TX, USA) overnight at 4˚C. The membranes were washed three times and incubated with secondary antibody horseradish peroxidase (HRP)-conjugated goat anti-rabbit

Table I. Blood lipids levels in group A and B rabbits.

Treatment group	HDL (mmol/l)	LDL (mmol/l)	TG (mmol/l)	CHOL (mmol/l)
Group A				
Week 0	0.42±0.07	0.31±0.05	0.78±0.09	0.97±0.07
Week 4	0.56±0.15	0.40±0.08	0.92±0.16	1.15±0.14
Week 8	0.49±0.08	0.37±0.18	0.59±0.24	0.81±0.03
Week 12	0.48±0.03	0.45±0.12	1.54±0.10	1.27±0.01
Group B				
Week 0	0.41±0.04	0.32±0.02	0.76±0.08	0.59±0.08
Week 4	6.65±0.91[a]	31.10±12.47[a]	0.79±0.36	33.48±8.07[a]
Week 8	9.00±3.00[a]	47.96±10.28[a]	1.69±1.37	40.26±2.96[a]
Week 12	10.93±0.13[a]	50.80±2.25[a]	4.22±2.88[a]	41.27±0.93[a]

[a]P<0.05 vs. group A at equivalent week. Data are expressed as the mean ± standard deviation (n=3). HDL, high density lipoprotein; LDL, low density lipoprotein; TG, triglyceride; CHOL, cholesterol.

IgG (cat. no. ab97051; 1:5,000) and goat anti-mouse IgG (cat. no. ab97023; 1:10,000) (both from Abcam) for 2 h at room temperature. Membranes were washed four times with TBST for 40 min. Proteins were visualized using Enhanced Chemiluminescence reagents (GE Healthcare Life Sciences) and densitometry was performed using Quantity One software 4.2 (Bio-Rad Laboratories, Inc.).

Statistical analysis. Differences between intra- and extra-cranial cerebral atherosclerosis were evaluated by Student's t-test for normally distributed data, or the Mann-Whitney rank sum test for nonparametric data. Data are expressed as the mean ± standard deviation. P<0.05 was considered to indicate a statistically significant difference.

Results

Lipid profile. Group B rabbits were fed an atherogenic diet. Mean cholesterol (CHOL), low density lipoprotein (LDL), high density lipoprotein (HDL) and triglyceride (TG) levels (mmol/l) were increased in group B compared with group A at the end of week 12 as follows: CHOL increased from 1.27±0.01 (Group A) to 41.27±0.93 (Group B), LDL from 0.45±0.12 to 50.80±2.25, HDL from 0.48±0.03 to 10.93±0.13, and TG from 1.54±0.10 to 4.22±2.88. Various lipid levels in different groups were revealed to increase over time in group B; in particular, LDL and CHOL levels were markedly increased. In group A, blood lipids remained within the healthy range. A statistically significant difference was observed in lipid levels between the two groups (P<0.05; Table I). These results suggested that an atherogenic diet resulted in hyperlipidemia.

Pathological sections. Pathological HE-stained sections of the CCA in groups A (Fig. 1A; magnification, x4) and B (Fig. 1B; magnification, x4), and of the MCA in groups A (Fig. 1C; magnification, x20) and B (Fig. D; magnification, x4) revealed that the degree of AS lesions was increased in the CCA compared with the MCA between different groups at week 12.

Table II. Comparison of cerebral artery diameters in different groups.

Treatment group	Common carotid artery diameter (μm)	Middle cerebral artery diameter (μm)
Group A		
Week 4	123.0±5.77	31.6±1.3
Week 8	129.3±13.4	36.1±2.6
Week 12	150.2±0.5	39.4±4.9
Group B		
Week 4	166.5±5.97[a]	58.9±2.04[a]
Week 8	176.4±6.6[a]	72.2±2.9[a]
Week 12	199.9±18.6[a]	81.1±2.3[a]

[a]P<0.05 vs. group A at equivalent week. Data are expressed as the mean ± standard deviation (n=3).

Therefore, a simple high-fat diet may lead to the formation of AS further aggravate the degree of AS lesions.

Comparison of intra- and extracranial cerebral artery segments. Previous reports have indicated that IMT measurements are a representative end point for AS and vascular disease risk (16). IMT measurements provide data on the efficacy of novel lipid-modifying techniques following a high-fat diet. To estimate AS progression between groups A and B, cross-sectional standardized IMT measurements were used, and because measurements were standardized, AS progression estimates were extrapolated from the cross-sectional data for each group. The IMT of the CCA and MCA were measured in all animals and combined to a per-subject average. IMT of the CCA and MCA increased over the 12 weeks in both groups; however, after 4, 8 and 12 weeks of receiving the different diets the CCA and MCA IMT was significantly increased in group B compared with group A (P<0.05; Table II). Thus, a high-fat diet can cause IMT thickness in different cerebral arteries.

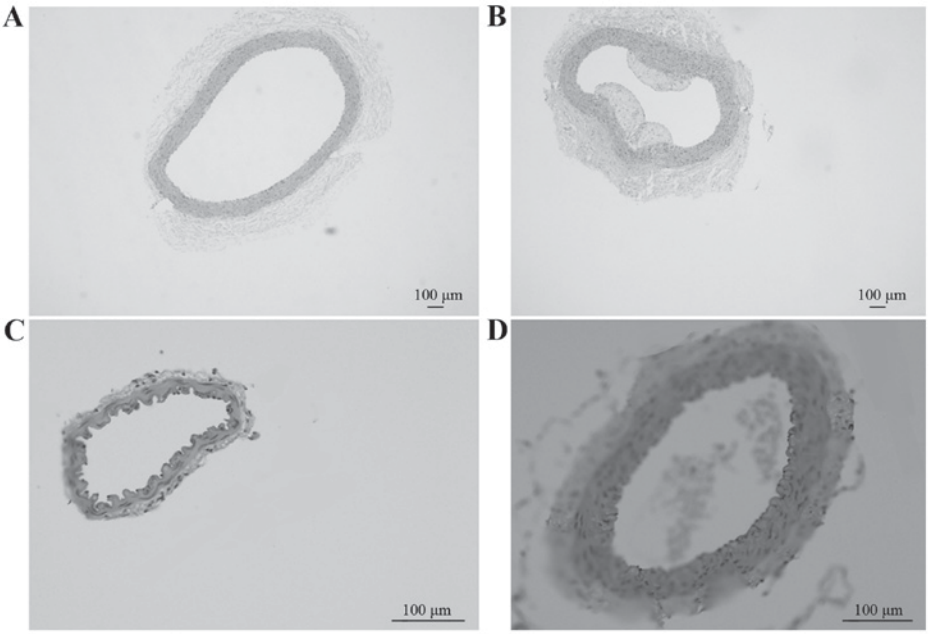

Figure 1. Hematoxylin-eosin-stained sections of arteries at week 12. Common carotid artery sections from (A) group A demonstrated smooth endomembrane and (B) in group B fatty streak formation plaque could be observed (magnification, x4). Middle cerebral artery sections from (C) group A had smooth endomembrane and (D) in group B the inhomogeneous intima-media was thickened (magnification, x20). Scale bar, 100 μm. Group A, control group; group B, high-fat diet group.

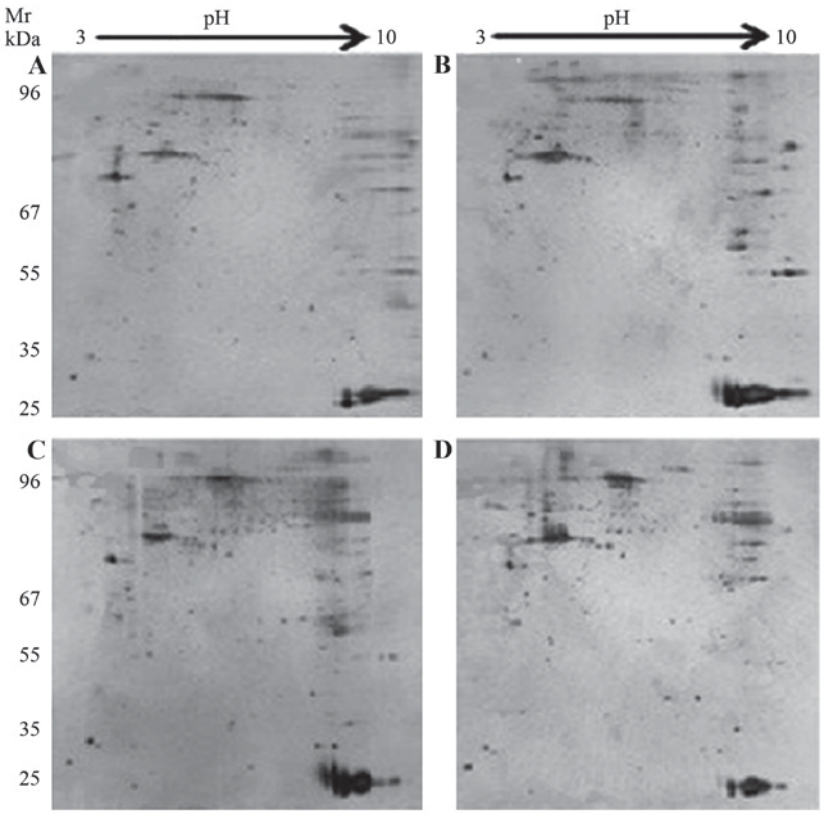

Figure 2. A representative 2-D gel map of CCA and MCA sections from groups A and B. Total protein extracts from (A) CCA in group A, (B) CCA in group B, (C) MCA in group A and (D) MCA in group B were separated on pH 3-10 nonlinear immobilized pH gradient strips in the first dimension followed by SDS-PAGE in the second dimension. The gels were visualized. In total, 8 altered spots were identified by 2-D gel electrophoresis and mass spectrometry analyses. Data on each of these protein species is reported in Table III. CCA, common carotid artery; MCA, middle cerebral artery; group A, control group; group B, high-fat diet group.

Proteomic analysis. To investigate the key proteins associated with ICAS and ECAS, 2-DE and silver staining were performed. 2-D gel images of proteins isolated from the MCA and CCA of groups A and B are presented in Fig. 2. Total

Table III. Differentially expressed proteins in intra- and extracranial cerebral arteries.

Spot no.[a]	Accession no.	Protein ID[b]	kDa	Mascot score
3203	gi126723746	Precursor protein	70.861	60
2902	gi148594078	Heat shock protein 70	71.424	105
4201	gil49864	α-smooth muscle actin	38.016	107
5001	gil291414651	β-galactose binding agglutinin	15.170	118
202	gil4507651	Tropomyosin α -4 chain isoform 2	28.619	91
2901	gil435476	Cell keratin 9	62.320	113
1203	gi112448	Tropomyosin α-1 chain	32.233	49
8201	gil395805236	Singleoctylic acid glyceride β2	69.636	53

[a]Spot no. corresponds to those presented in Fig. 2. [b]Protein ID confirmed by peptide mass fingerprinting.

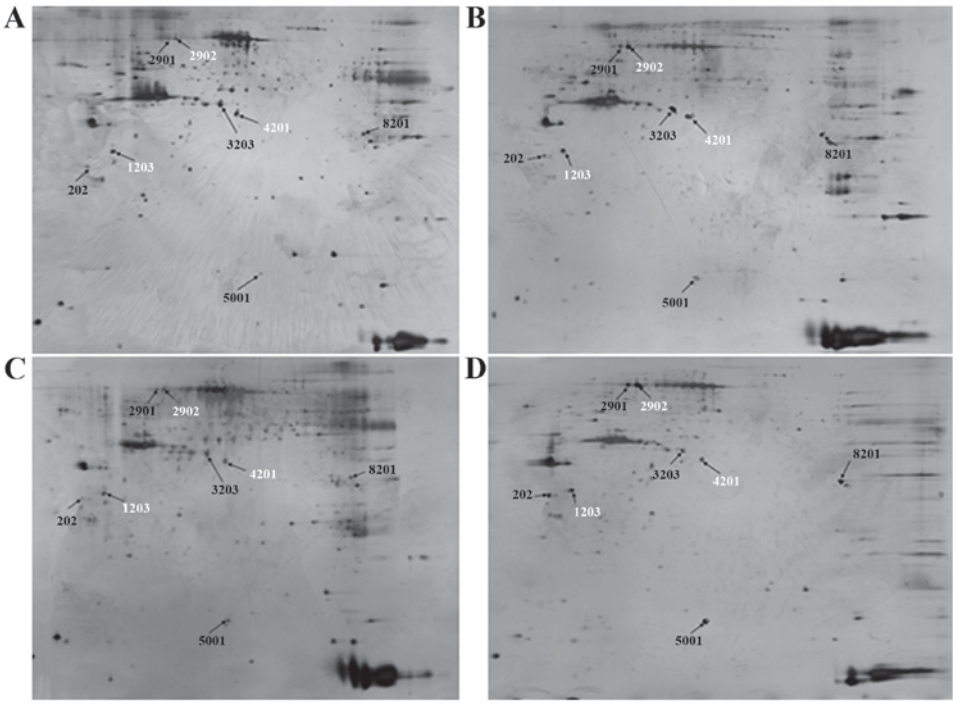

Figure 3. Protein expression differences in groups A and B, as analyzed by mass spectrometry. (A) CCA in group A, (B) CCA in group B, (C) MCA in group A and (D) MCA in group B. Black arrows indicate proteins that were significantly altered following a high-fat diet; >439 protein spots were detected per gel. Of the altered proteins, 25 protein expression levels were different and 8 spots (marked with arrow and number) demonstrated significant alterations and were quantitatively high enough to be identified by Matrix-assisted laser desorption/ionization-time-of-flight tandem mass spectrometry. CCA, common carotid artery; MCA, middle cerebral artery; group A, control group; group B, high-fat diet group.

protein extracts from different groups were separated on 1-D nonlinear IPG strips (pH 3-10) followed by 2-D SDS-PAGE. Spectrum analysis was used to identify protein spots, with >2-fold or <0.5-fold-changes as a standard. Per gel, >439 protein spots were detected, and well-matched spots and control gels were analyzed for comparative proteomics. Of the altered proteins, 25 protein expression levels had significant alterations, and were quantitatively increased enough to be identified by matrix-assisted laser desorption/ionization (MALDI)-TOF/TOF. MS data were analyzed using the Mascot search engine. All 25 spots were identified with high confidence by comparing >70% different degree groups. The present study identified that protein expression levels of 25 proteins were significantly increased in the MCA and CCA of group B compared with group A using Mascot retrieval software. A total of 8 differential proteins were observed: Precursor protein (albumin A chain), TPM1, HSP70, α-smooth muscle actin, β-galactose binding agglutinin, TMP4 isoform 2, cell keratin 9 and single octylic acid glyceride-β (Fig. 3; Table III).

Western blot analysis. Based on the fold-change results and the protein biological functions, the following proteins were selected for further evaluation by western blotting: TPM1, HSP70 and α-smooth muscle actin. GAPDH served as an internal control. Western blot images of protein expression levels of TPM1, α-smooth muscle actin and HSP70 from MCA and CCA in groups A and B are presented in Fig. 4A. Protein expression was semi-quantitatively evaluated using

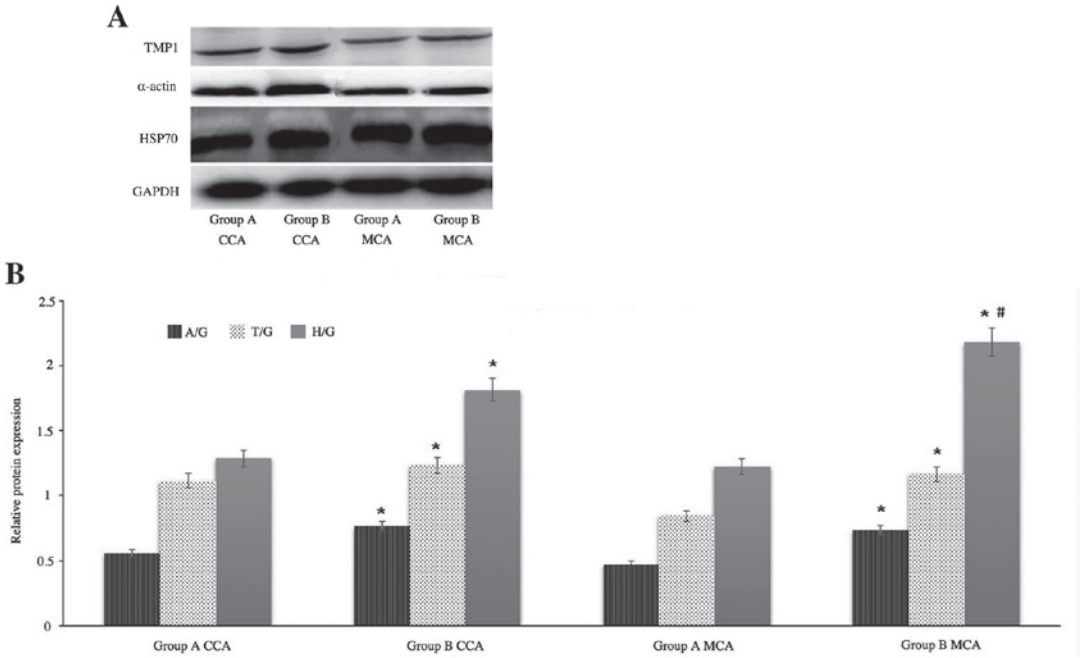

Figure 4. Western blot analysis. (A) Representative western blot images and (B) quantification of TPM1, α-smooth muscle actin and HSP70 protein expression levels in the CCA and MCA of groups A and B. GAPDH served as an internal control. Data are expressed as the mean ± standard deviation from two experiments. *P<0.05 vs. group A CCA and #P<0.05 vs. group B CCA. TPM1, tropomyosin α-1 chain; HSP70, heat shock protein 70; α-actin, α-smooth muscle actin; CCA, common carotid artery; MCA, middle cerebral artery; group A, control group; group B, high-fat diet group; A/G, α-smooth muscle actin/GAPDH; T/G, TPM1/GAPDH; H/G, HSP70/GAPDH.

densitometry (Fig. 4B). The results revealed that the expression of α-smooth muscle actin and TPM1 chain from CCA in group B are increased marginally compared with from MCA in group B, however, the expression of HSP70 from MCA in group B is significantly increased compared with from CCA in group B. It was consistent with the results of mass spectrum identification.

Discussion

Biomarkers including C-reactive protein, B-typenatriuretic peptides and cardiac troponins have previously been used by clinicians as predictors of future cardiovascular events (17,18). However, highly reliable biomarkers of cerebrovascular events remain to be identified. Existing proteomic studies on nervous system disease have primarily focused on penumbral phenomena, movement disorders and neurodegenerative disease (19-23). In the present study, a proteomics approach was used to identify if protein expression levels were altered in response to ICAS and ECAS based on the controversy between hyperlipidemia and cerebral atherosclerosis. Previous studies have investigated the differences in risk factors and stroke mechanisms between ICAS and ECAS, and demonstrated that hyperlipidemia is more closely associated with ECAS (2), whereas hyperlipidemia was more closely associated with MCA stenosis (5). The differences of protein expressions in high-fat-fed rabbits between ICAS and ECAS are still unclear. In order to provide information about the possible mechanisms of atherosclerosis progression and identify potential therapeutic target molecules for ICAS and ECAS, a global proteomic analysis of different cerebral arteries was performed in the present study.

The present study demonstrated that mean CHOL, LDL, HDL and TG levels significantly increased by week 12 following a high-fat diet compared with rabbits that had received a normal diet. Lipid levels were increased over time in group B compared with group A; in particular, LDL and CHOL levels were markedly increased. It has previously been reported that IMT measurements are a representative end point for AS and vascular disease (16). To estimate AS progression following a high-fat diet, IMT of the CCA and MCA were measured in all rabbits. At week 12, the IMT and AS degree of CCAs and MCAs was significantly increased in group B compared with group A. These data indicated the successful establishment of a cerebral AS model of rabbits with a high-fat diet, and that hyperlipidemia is positively associated with intra- and extracranial cerebral AS. Proteomic analysis and comparison of intra- and extracranial cerebral AS responses to hyperlipidemia in rabbit was performed by 2-DE and MS analyses. The present study identified an average of 439 different proteins in the analyzed samples; 25 proteins were differentially expressed in group A compared with group B, and 8 spots were significantly different; these 8 spots were quantitatively increased enough to be identified by MALDI-TOF/TOF analysis. Only three of the significantly expressed proteins (TPM1, HSP70 and α-smooth muscle actin) were examined by western blot analysis due to limited antibody sources.

α-smooth muscle actin is a component of the contractile apparatus in muscle cells, and is an important marker of AS plaque formation. Loss of α-smooth muscle actin reduces the contractile ability of the cell, increases smooth muscle actin proliferation and leads to excessive neointimal formation with vascular injury (24,25). The majority of upregulated

α-smooth muscle actin expression may be involved in early remodeling that occurs prior to marked adaptive alterations (26). The present study demonstrated the upregulation of α-smooth muscle actin expression in ICAS and ECAS in rabbits that received a high-fat diet compared with rabbits on normal diet, and the expression of α-smooth muscle actin from extracranial cerebral arteries was significantly increased compared with intracranial cerebral arteries. Increased expression levels of α-smooth muscle actin following a high-fat diet may enhance inflammation of injured vessels, leading to proliferation of vessel smooth muscle actin. The increase of vessel smooth muscle actin and intimal hyperplasia of arteries is the primary pathological event leading to stenosis.

Simoneau *et al* (27) demonstrated that TPM1 may regulate endothelial barrier integrity in response to oxidative stress conditions, and phosphorylation of TPM1 at Ser283 regulates endothelial permeability and transendothelial migration of cancer cells. The primary finding of the present study was that TPM1 expression levels were abundantly increased in CCAs and MCAs by a high-fat diet. TPM1 expression was semi-quantitatively evaluated using densitometry, and the results demonstrated that the expression of TPM1 in CCA was increased marginally compared with MCA in group B; the average intensity of TPM1/GAPDH in CCA was 1.232 and the average intensity of TPM1/GAPDH in MCA was 1.164, which indicates that extracranial cerebral arteries are more susceptible to endothelial damage in AS than intracranial cerebral arteries.

HSP70 has been demonstrated to suppress inflammation and tissue damage via an underlying mechanism that involves an enhanced regulatory response mediated by antigen-specific interleukin-10 production (28). Overexpression of HSP70 serves an important role in cell death and has neuroprotective effects via an anti-inflammatory underlying mechanism, and has been demonstrated to be positively associated with ICAS and ECAS (29,30). However, whether ICAS or ECAS is more closely associated with altered expression levels of HSP70 remains to be elucidated. Furthermore, expression levels of HSP70 from intracranial cerebral arteries were significantly increased compared with extracranial cerebral arteries in this study, in contrast to the expression patterns of TPM1 and α-smooth muscle actin. Therefore, HSP70 may have AS-protective properties via anti-inflammatory underlying mechanisms in ICAS compared with ECAS. This finding is consistent with a previous study, which examined the proteomic analysis of stable and unstable carotid atherosclerotic plaques (6). The findings of the present study may partly explain previous contradictory observations on the differences in cerebral AS mechanisms.

The present study demonstrated that hyperlipidemia exerts varying influences on intra- and extracranial cerebral arteries. Proteomic analysis revealed that 8 proteins exhibited varying degrees of differential expression in intra- and extracranial cerebral arteries, which are involved in oxidative stress, tumor metastasis, inflammation, cholesterol transport, cell apoptosis and adhesion. Alterations in protein expression levels of α-smooth muscle actin, TPM1 and HSP70 may be involved in the differences between ICAS and ECAS. The underlying mechanisms of these proteins on the formation of AS require clarification. Further investigating the pathogenesis of differential protein expression levels between intra- and extracranial cerebral arteries may facilitate the identification of novel biological markers for the diagnosis and treatment of cerebral arteriosclerosis.

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