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Original Article

Variations of rats' telomere lengths and gene expression levels of telomerase and telomere-binding proteins in an intracranial aneurysm model

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Abstract: An intracranial aneurysm (IA) is a leading contributory factor in subarachnoid haemorrhage (SAH), while its etiological mechanism has yet to be revealed. Meanwhile, although telomeres have proved to be critical roles in onsets of multiple neurological disorders, whether telomeres are connected with IAs or not remains to be unreleased. The purpose of this study is to dig up the mechanism how related risk factors contribute to IAs. In a bid to figure it out, we constructed an IA rat model and then investigated variations of telomere lengths, telomerase and telomere-binding protein expression levels therein. Adult female Sprague-Dawley (SD) rats were initially treated with ligation of left common carotid arteries and bilateral renal arteries and bilateral oophorectomy. After a week of rehabilitating feeding, they were fed with feedstuff containing sodium chloride (8%) and L-methionine (3%). Through the first six months of modelling, lengths of telomeres in peripheral leucocytes were measured monthly. In the sixth postoperative month, blood pressure levels and concentrations of homocysteine (Hcy) and estradiol (E2) in serum were measured. And then, rats had been killed before Willis' circles were taken out. Afterwards, telomere lengths and gene expression of telomerase and telomere-binding proteins in vascular tissues were detected. QPCR analyses show that, within the first six months of modelling, telomeres in peripheral leucocytes shortened at first, then lengthened glacially, and finally shortened sharply. In the sixth month of modelling, lengths of telomeres in rat cerebrovascular tissues in the model group were significantly shorter than those in the control group, and lengths of telomeres in peripheral leucocytes were positively correlated with lengths of telomeres in rat cerebrovascular tissues in the model group. In addition, in rat cerebrovascular tissues, the expression level of telomerase reverse transcriptase (TERT) in the model group was much lower than that in the control group. In the meantime, the expression levels of telomere-binding proteins including TRF2 and TPP1 in the model group were far higher than those in the control group, while the comparison of expression levels of other telomere-binding proteins including POT1, TIN2, TRF1, and RAP1 between two groups is not statistically significant. In conclusion, outcomes of our experiments indicate that formation of IAs is very likely associated with the increased expression levels of telomere-binding proteins including TRF2 and TPP1 which inhibit telomerase activity, and, further, impair the recovery of the telomere-telomerase system to accelerate the shortening of telomeres.

Keywords: Intracranial aneurysm, telomere, telomerase, telomere-binding protein, animal model

Introduction

The IA is a disease that can induce serious cerebral stroke incident, the population incidence is 5-10% [1], in case the aneurysm ruptures, it will bring serious consequences to individuals, families and even society. However, its etiological mechanism still needs to be uncovered till now.

Recently, several meta-analyses demonstrate that there is a negative correlation between incidences of assorted cardiovascular or cerebrovascular strokes, such as coronary diseases, myocardial infarctions, and ischemic strokes, and lengths of relevant telomeres [2]. Accordingly, we shifted our focus onto telomeres when it came to the study on the etiological mechanism of IAs. Specifically, we re-exam-

ined IAs from the perspective of telomere damage and repair. Having reviewed masses of archives simply concerning IAs and telomeres, we were astonished by the fact that the onset and exacerbation of IAs and the truncation of telomeres share miscellaneous risk factors. For example, both of them correlate closely with some individual characteristics, such as inheritance, gender, age, smoking, and obesity, as well as some diseases, such as atherosclerosis (AS) and hyperhomocysteinaemia (HHcy). Under the influence of those shared risk factors, the onset and exacerbation stages of IAs coincide perfectly with the trend of telomere lengths [3-5]. Thus we assume that telomeres serve as the same important actor in the onset and exacerbation of IAs as they do in cardiovascular or cerebrovascular strokes.

Telomere are TTAGGG cap structures of repetitive sequences at the chromosome termini of eukaryotic cells. Research has confirmed that they play a vital role in the process of protecting chromosomes and stabilizing the genetic system. Because these cap structures are truncated during cell division and when their lengths shorten to a certain point, cells fail to divide and proliferate such that they enter cellular senescence and apoptosis, intervention of telomere lengths is particularly important [6].

Regulation of telomere lengths relies upon the synergy of telomerase and telomere-binding proteins. Telomerase is a kind of ribonucleoproteins (a protein-RNA complex) that can promote the elongation of telomere termini. It consists of a telomerase RNA molecule, relevant proteins, and TERT. It can maintain the stability of telomere lengths because it carries its own RNA molecule which is used as a template when it synthesizes telomere DNA strands via reverse transcription. The role of TERT is catalysing the process of reverse transcription [7, 8]. Studies identify that as TERT's mRNA strand is only transcribed in telomerase-positive tissues, transcription of TERT is a prerequisite for activation of telomerase [9]. Moreover, the expression level of TERT's mRNA rises while telomerase is becoming increasingly active and their linear trends are almost paralleled [10]. Therefore, practically, we can detect telomerase's activity by virtue of measuring TERT's transcription levels, and, further, evaluate effects of relevant risk factors on the telomere regulation mechanism.

Telomere-binding proteins are core components that mediate biological functions of telomeres. Not only are they expressed nearly nowhere but at chromosome termini, but also their scope of functions is confined to telomeres [11]. Mammalian somatic cells contain six telomere-binding proteins: Telomere Repeat Factor 1 (TRF1), Telomere Repeat Factor 2 (TRF2), Protection of Telomere 1 (POT1), Adrenocortical Dysplasia Protein Homolog (ACD gene, aka TPP1), TRF1- and TRF2-Interacting Nuclear Protein 2 (TIN2), and Repressor/Activator Protein 1 (RAP1). They bind to each other to assemble the shelterin complex which regulates telomere lengths to a great extent through binding of telomerase and telomeres or direct induction of DNA damage [12]. Besides, their respective unique structures allow them to regulate telomere functions in various ways individually or collectively [13]. Hence, we can conduct a more intensive analysis of profound effects of risk factors on the telomere regulation mechanism by means of probing into these telomere-binding proteins' expression levels.

To sum up, in the context of our newly built IA rat model, variations of telomere lengths and expression levels of telomerase and telomere-binding proteins are discussed in this study, which is likely to disclose the inherent mechanism of how risk factors intervene in the formation of rats' IAs, thereby laying the scientific and reliable foundation for further exploration into the etiological mechanism of IAs based upon the telomere damage and repair theory.

Materials and methods

This experiment was scrutinized and authorized by the Ethics Committee of the Affiliated Hospital of the Logistics University of PAP and was carried out following the guide from the National Institutes of Health for the Care and Use of Laboratory.

IA model and treatment

24 8-to-10-week-old CL female SD rats (250 to 300 g) were selected for the study (source: Laboratory Animal Centre at Academy of

Table 1. Primers used in this study

Primer Name	Primer sequence (5'-3')		Final concentration
TEL	Forward	GGTTTTTGAGGGTGAGGGTGAGGGTGAGGGTGAGGGT	100 nmol/L
	Reverse	TCCCGACTATCCCTATCCCTATCCCTATCCCTATCCCTA	900 nmol/L
TERT	Forward	AGTGGTGAACTTCCCTGTGG	400 nmol/L
	Reverse	CAACCGCAAGACTGACAAGA	400 nmol/L
TRF-1	Forward	TACCAAACTCAAGCCCCATC	400 nmol/L
	Reverse	GCAGCAAACTCACATCGAAA	400 nmol/L
TRF-2	Forward	AGAAGAAAGCGAGTGGGTGA	400 nmol/L
	Reverse	TTGTGAGTCCTGTGGCTCTG	400 nmol/L
POT-1	Forward	CAGATTCGGCAGTCACTCAA	400 nmol/L
	Reverse	CTTCAAAACGGCACACAATG	400 nmol/L
TPP-1	Forward	CAGTGACCACCCAGGACTTT	400 nmol/L
	Reverse	CAACAGGTCCCACTCCTTGT	400 nmol/L
TIN-2	Forward	AAAACCAGCATCCCACAGTC	400 nmol/L
	Reverse	ATGGTAGGCCTGTGTTCCTG	400 nmol/L
RAP-1	Forward	GTGAGCCTTGGTTGGAATGT	400 nmol/L
	Reverse	CCTGGGGAATGGGATAGTTT	400 nmol/L
GAPDH	Forward	TACACTGAGGACCAGGTTG	400 nmol/L
	Reverse	CCCTGTTGCTGTAGCCATA	400 nmol/L
AT1 (DNA reference)	Forward	ACGTGTTCTCAGCATCGACCGCTACC	400 nmol/L
	Reverse	AGAATGATAAGGAAAGGGAACAAGAAGCCC	400 nmol/L

Military Medical Sciences, license No.: SCXK-(Jun) 2012-0004). Per four rats were caged as a unit under environmentally controlled conditions in a 12-hour light/dark cycle. They were all supplied with a normal diet as adaptive feeding for 1 week. Before modelling, rats were randomly divided into two groups: the model group and the control group. Initially, they were all intraperitoneally (ip.) injected to anaesthesia with 1% pentobarbital sodium (40 mg/kg, doses increased p.r.n.). Then, rats' left common carotid arteries and posterior branches of bilateral renal arteries in the model group were ligated and their bilateral ovaries were excised. After a week of rehabilitating feeding, they were given ad libitum access to a diet with sodium chloride (8%) and L-methionine (3%). Correspondingly, rats' relevant vessels and ovaries in the control group were simply exposed to air for 20 minutes and then incisions were sutured. They were fed with a normal diet ad libitum. Anorectic and dead rats were replaced with new ones within 3 postoperative days.

Measurement of blood pressure

In the sixth month of modelling, rats' caudal-arterial systolic pressure levels were measured in a conscious state by use of rat-tail non-invasive blood pressure monitors. For the purpose of enabling rats to adapt to the measurement condition and preventing rats from struggling and fluctuations of blood pressure, blood pressure levels were measured once a day in 3 consecutive days and the third-day results were noted down as valid data.

Blood sample collection and extraction of genomic DNA and intact RNA

200 μL blood samples were collected from each rat's retro-orbital venous plexuses and stored in centrifugal tubes before modelling and at the beginning of every month till the sixth month of modelling. Genomic DNA was extracted from blood samples following methods and procedures described on kit instructions, and used for measuring telomere lengths and expression levels of TERT and telomere-binding proteins in peripheral leucocytes.

Meanwhile, in the sixth month of modelling, 1 mL blood samples were collected from each rat's retro-orbital venous plexuses and stored in coagulative tubes. They were delivered to the clinical lab in our hospital and concentrations of E2 and serum Hcy were tested there

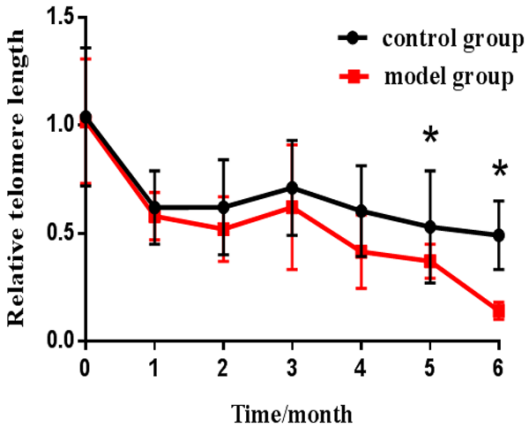

Figure 1. Changes of telomere lengths in peripheral leucocytes during the 6 months. *P < 0.05 versus the Control group.

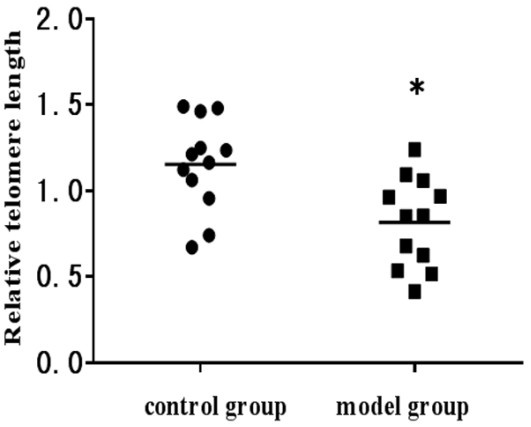

Figure 2. The relative lengths of telomere in cerebrovascular tissues. *P < 0.05 versus the control group.

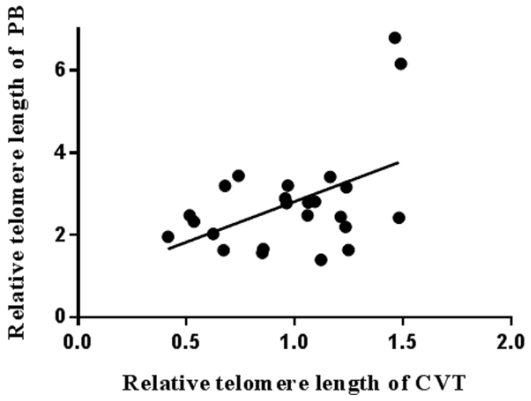

Figure 3. Correlation between lengths of telomere in peripheral leucocytes and in cerebrovascular tissues (n = 24, r = 0.475, P = 0.019).

(MINDRAY automatic biochemical detection system, BS-480).

Extraction of intact RNA and DNA in cerebrovascular tissues

In the sixth month of modelling, rats were killed by cervical spinal interruption. Next, Willis' circles were detached from rats' skull bases and placed in 1.5 mL sterile centrifugal tubes. Then, 350 μL lysate RLplus (β-mercaptoethanol, i.e. BME, added as required) was added into each centrifugal tubes. Tissues were ground by the tissue mill at 3000/min for 6 minutes, and then centrifuged at 12000 rpm for 3 to 5 minutes. Afterwards, the supernatant was carefully collected to DNA adsorption columns, CB3s placed in 2 mL centrifugal tubes (12000 rpm, 30 to 60 s). Filtrate was collected and CB3s used before were stored in collecting tubes at room temperature. Finally, intact RNA and genomic DNA were extracted according to methods and procedures described on kit instructions for the use of testing cerebrovascular telomere lengths and expression levels of TERT and telomere-binding proteins.

Assays concerning telomere length and relative expression levels of TERT and telomere-binding proteins

A 25 μL reaction system was established and each sample was prepared in triplicate. The entire set was processed through steps of: 1. 10-minute initial denaturation at 95°C; 2. 40 cycles of 15-second denaturation at 95°C and 1-minute annealing at 60°C. Then the gradient of temperature increase was controlled at 0.3°C while melting curves were being analysed (**Table 1**). Quantification of target genes was achieved through the 2-ΔΔCt method. Telomere length was measured according to Cawthon's research, which firstly calculated the ratio of telomere gene to intrinsic gene expression in each sample ($[2^{Ct(tel)}/2^{Ct(AT1)}]^{-1}=2^{-\Delta Ct}$), and secondly calculated the T/S values ($T/S = 2^{-\Delta Ct}/2^{-\Delta Ct(average\ of\ control\ group)}$). Average T/S is expected to be proportional to the average telomere length per cell. Samples with a T/S > 1.0 have an average telomere length greater than that of the standard DNA; samples with a T/S < 1.0 have an average telomere length shorter than that of the standard DNA [6].

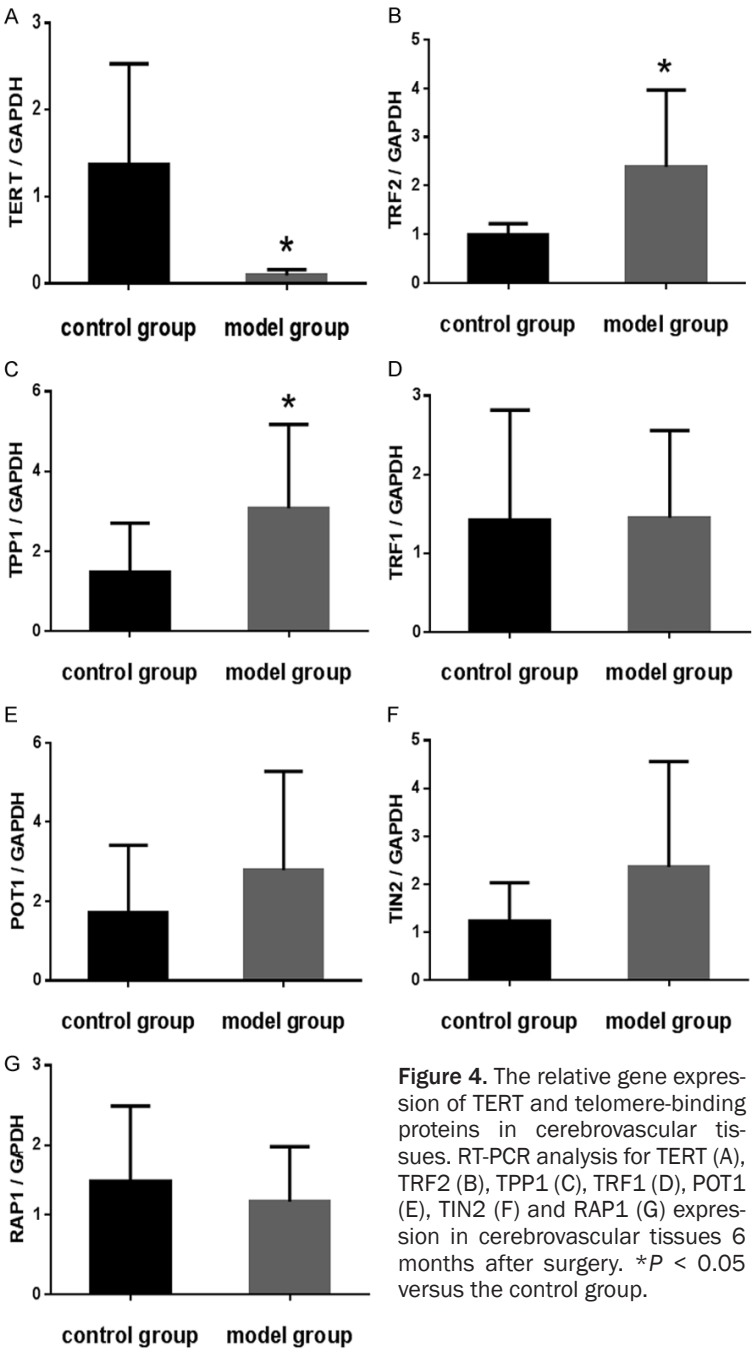

Figure 4. The relative gene expression of TERT and telomere-binding proteins in cerebrovascular tissues. RT-PCR analysis for TERT (A), TRF2 (B), TPP1 (C), TRF1 (D), POT1 (E), TIN2 (F) and RAP1 (G) expression in cerebrovascular tissues 6 months after surgery. *P < 0.05 versus the control group.

Statistical analyses

SPSS 17.0 was used for data analyses and all data are presented as mean ± standard deviation (SD). Unpaired t-tests were applicable only when two sets of samples are normally distributed and share the equal variance. Otherwise, nonparametric Kruskal-Wallis tests were adopted. The Pearson correlation coefficient is the measure of data's correlation. P values of less than 0.05 were considered statistically significant.

Results

Levels of blood pressure, serum Hcy, and E2

Rats' blood pressure level in the model group was significantly higher than that in the control group (189.44 ± 16.45 mmHg vs. 133.49 ± 15.01 mmHg). The serum Hcy level in the model group was significantly higher than that in the control group (50.06 ± 7.35 mmol/L vs. 13.48 ± 3.22 mmol/L). The E2 level in the model group was significantly lower than that in the control group (90.6 ± 8.45 pmol/L vs. 181.33 ± 12.90 pmol/L). Variances mentioned above are all statistically significant (P < 0.05).

Lengths of telomere in peripheral leucocytes

The variance of initial lengths of telomeres in rats' peripheral leucocytes in two groups is not statistically significant (P > 0.05). After a month of modelling, compared with initial lengths, telomeres were significantly shortened respectively (P < 0.05), but the comparison between two groups is not statistically significant (P > 0.05). After two to three months of modelling, telomeres in two groups were all slightly elongated, but the comparison between two groups is still not statistically significant (P > 0.05). Since the fourth month, telomeres in the model group had been sharply truncated. In the fifth month, telomeres in the model group were significantly shorter than those in the control group (P < 0.05) (**Figure 1**).

Lengths of telomere in cerebrovascular tissues

In the sixth month, lengths of rats' telomeres in cerebrovascular tissues in the model group were significantly shorter than those in the control group (0.81 ± 0.26 vs. 1.15 ± 0.27, P < 0.05) (**Figure 2**).

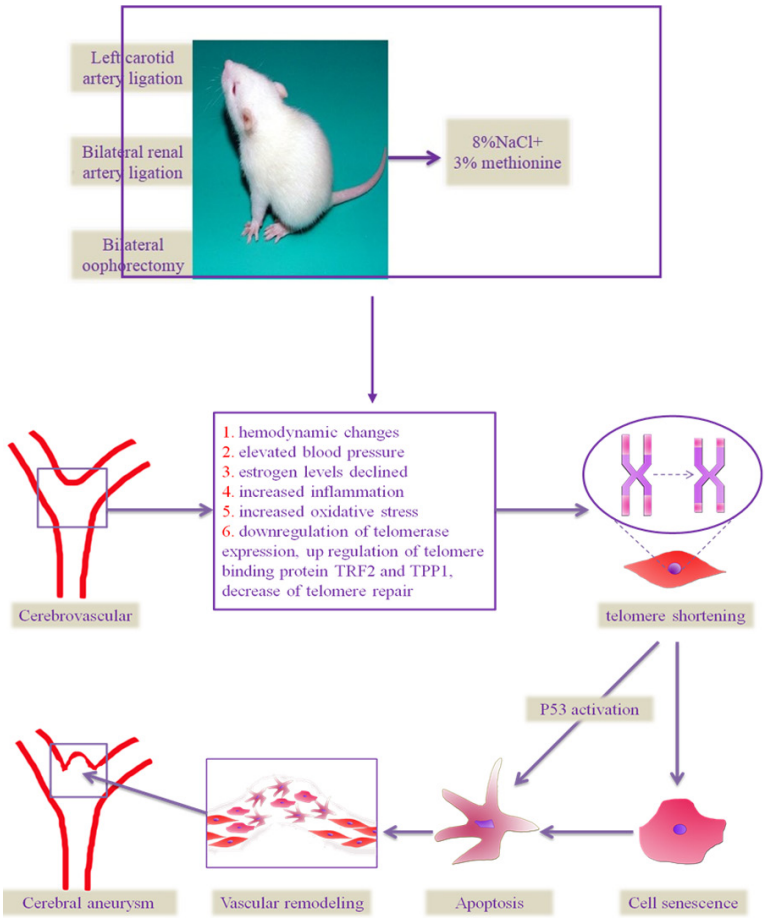

Figure 5. IA modelling and the hypotheses of the IA mechanism.

Correlation between lengths of telomere in peripheral leucocytes and in cerebrovascular tissues

In the sixth month, relative lengths of telomeres in two groups were recalculated respectively and the average length of cerebrovascular telomeres in the control group was set as the benchmark. According to the Pearson correlation coefficient, lengths of telomeres in peripheral leucocytes and in cerebrovascular tissues are significantly correlated (r = 0.475, P = 0.019) (**Figure 3**).

Expression of tert in cerebrovascular tissues

In the sixth month, the expression level of TERT in cerebrovascular tissues in the model group was significantly lower than that in the control group (0.09 ± 0.07 vs. 1.37 ± 1.16, P < 0.05) (**Figure 4**).

Expression of telomere-binding proteins in cerebrovascular tissues

In the sixth month, expression levels of TRF2 and TPP1 in the model group were significantly higher than those in the control group (P < 0.05). Expression levels of POT1 and TIN2 in the model group were higher than those in the control group, but not statistically significant. Expression levels of TRF1 and RAP1 in two groups were not significantly divergent (P > 0.05) (**Figure 4**).

Discussion

According to analyses of baseline indicators concerned, rats' caudal-arterial systolic pressure level and serum Hcy level in the model group steadily rose to 180 mmHg and 45 μmol/L respectively and were significantly higher than those in the control group. Meanwhile, the serum E2 level in the model group steadily dropped lower than 100 pmol/L and was significantly lower than that in the control group. Results above indicate that risk factors significantly intervene in the etiological mechanism, and, further, this IA model is stable and repeatable.

Judged from dynamic surveillance of telomere lengths in peripheral leucocytes, curves of telomere lengths for both groups follow the 'descending-ascending-descending' trend. Research identifies that moderate-severe trauma remarkably truncates telomeres in peripheral leucocytes over a short period of time [14]. Also, another study verify this statement in on variations of telomere lengths in peripheral leucocytes targeted at traumatic brain injury [15]. Accordingly, we assume that, in the first postoperative month, telomeres in peripheral leucocytes in two groups shortened sharply because rats' automatic reaction to acute traumatic lesions activated their stress state. Similarly,

through the second and the third month, activation of the telomere repair mechanism following stress contributed to the glacial compensatory elongation of telomeres in rats' peripheral leucocytes. However, rats and humans are distinct at this stage. Humans' telomeres in peripheral leucocytes elongate to pre-traumatic lengths promptly as lesions recover. As to rats, despite automatic recovery and compensatory elongation in both groups, it is a glacial and inefficient compensation process, particularly shown in the recovery level of the control group in this experiment. This variance presumably results from species diversity although specific reasons remain ambiguous. In the fourth month, telomeres in peripheral leucocytes in both groups began to shorten again. Lengths became divergent in two groups in the fifth month and diverged more markedly in the sixth month. We presume that this result is very likely connected with the synergistic effects of multiple risk factors relating to IAs in the model group. Piles of earlier researches buttress our assumptions. They confirm from different perspectives that hypertension, haemodynamic variations, HHcy and low levels of oestrogen can all incur truncation of telomeres in mammalian peripheral leucocytes [16-19].

In terms of cerebrovascular telomere lengths, overall, telomeres in the model group were significantly shorter than those in the control group in the sixth month of modelling. Combined with our earlier studies on the novel IA rat model (incidence of IAs: 142%, success rate of modelling: 100%), we suppose that telomere lengths are strongly related to formation of IAs. It is similar to the result of research on abdominal aortic aneurysms (AAAs) [20]. This research identifies that AAA patients' peripheral leucocytic telomeres and abdominal aorta vascular telomeres are all significantly shorter than those in AAA-free subjects. Another research on AAA also substantiates the claim that breakages of DNA strands rise dramatically when vascular smooth muscular telomeres in AAAs shorten significantly [21]. We thus speculate that the synergy of risk factors linked with IAs may damage telomeres on intracranial arterial walls. Damaged telomeres can directly induce expression of P53 and then activate apoptosis signalling pathways [22]. Apoptosis can also be achieved through telomere-mitochondrion pathways after mitochondrial dysfunction owing to inhibition of transcription and expression of mitochondrial oxidation respiratory chain-related proteins [23]. Activating other unknown signalling pathways can lead to cerebrovascular endothelial apoptosis and vascular remodelling as well. The final consequence of these reactions is inducing the formation and exacerbation of IAs.

For all the samples, lengths of telomeres in peripheral leucocytes and in cerebrovascular tissues are positively correlated ($r = 0.475$, $P = 0.019$), which suggests this correlation is not disturbed by risk factors concerning IAs. It is worth noting that this result is similar to the result of Wilson et al.'s research that telomere lengths on human AAA's vascular walls and in peripheral leucocytes are positively correlated [20]. Not only can this result confirm that lengths of telomeres in peripheral leucocytes indicate damage conditions of IA vascular walls and the degree of aging of holistic vessels, but it also makes it possible to replace cerebrovascular telomere lengths with peripheral leucocytic telomere lengths in the IA model. It effectively averts contradictions of methods and procedures between cerebrovascular telomere length measurement and cerebrovascular construction. However, given limitations stemming from the small sample size, r is relatively low in this experiment ($r = 0.5$).

As to expression levels of TERT in cerebrovascular tissues, the level in the model group was significantly lower than that in the control group in the sixth month, implicating that telomere expression was noticeably inhibited. From our point of view, synergistic effects of hypertension, HHcy, haemodynamic variations, and low oestrogen levels remarkably upregulate the level of oxidative stress, which produces excessive oxygen free radicals (OFRs). The free radical oxidation can convert 5'-guanine on the telomere repetitive sequence TTAGG to 7, 8-dihydro-8-oxoguanine (8-oxo-G) and lead to decrease in telomerase activity by directly hampering the binding of telomerase and telomeres [24]. This mechanism is considered the leading cause of the obvious inhibition of telomerase activity in the model group and the principal stimulus to rats' cerebrovascular telomere damages. However, some investigators unveil the fact that for hypertension patients, activity of telomerase rises both in peripheral leuco-

cytes and in vascular tissues [25]. It is inconsistent with our observation. The possible reason for this inconsistency is, as a single risk factor, although hypertension can trigger oxidative stress and further inhibit telomerase activity partially, it activates the repair mechanism of the telomere-telomerase system as well. Massively increased compensatory telomerase activity overwhelms inhibition rising from hypertension. In contrast, inhibition derived from the synergy of multi-factors overwhelms increased compensatory telomere expression, signifying a decline in activity as a whole. Therefore, it is a process of defeating and defeated amongst mechanisms in vivo.

Seen from expression levels of telomere-binding proteins in cerebrovascular tissues, in the sixth month, expression levels of TRF2 and TPP1 in the model group were significantly higher than those in the control group. They are considered to be implicated in telomere truncation and telomerase inactivation. TRF2 is an extremely sequence-conserved protein structure which plays a key role in recruiting the break repair protein, MRE11, and RAP1 to telomeres so as to prevent 3' ends of telomeres from losing and chromosome ends from joining [26, 27]. Earlier investigators claimed that TRF2 is a negative regulator of telomere lengths because telomeres are truncated by supplementing exogenous TRF2 whether in telomerase positive cells or in telomerase negative cells [28, 29]. As research delves deeply into this issue, investigators identified that when TRF2 is removed from cells or fragmented TRF2 is expressed, the chromosome ends joining and excessive loss of telomere DNA can also be observed [30]. It is widely acknowledged that both excessively high and extremely low levels of TRF2 expression can sabotage the integrity of telomere structures. In other words, only when the expression level is maintained normal can TRF2 be protective towards telomeres. TRF2 was excessively expressed in the model group in this experiment. As mentioned earlier, the synergy of risk factors linked with IAs such as hypertension, HHcy, haemodynamic variations and low oestrogen levels activates rats' oxidative stress mechanism in vivo. This mechanism acts on TRF2 and excessively upregulates TRF2 expression levels. Excessive expression of TRF2 allows TRF2 to bind with DNA strands at telomere termini at saturation point and promotes the formation of T-loops, structures that keep telomerase from closing to and binding to telomeres and, further, serve as a negative regulator of telomere lengths. A series of interactions mentioned above significantly affect integrity of telomeres and aggravate telomere damages. It is presumed to be another major mechanism that damages rats' telomeres.

TPP1 is another telomere-binding protein which was discovered to interact with TIN2. It not only binds with carboxyl termini of POT1 to form heterodimers that can enhance POT1 binding activity of telomeres, but also interacts with telomerase to regulate telomerase activity. Through these ways, TPP1 performs its protective function related to maintaining telomere integrity and length [31]. Meanwhile, it stabilizes the shelterin complex by interacting with TRF1 and TRF2 after binding with TIN2 [32]. The expression level of TPP1 in the model group was relatively high in this experiment. We suppose that as telomeres were truncated in the model group, organisms tended to increase telomerase activity and further repair damaged telomeres by mediating TPP1 expression. Whereas, the synergy of multiple risk factors inhibited telomerase activity significantly such that TPP1 was consistently mediated and thus high expressed as compensation. In the meantime, excessive expression of TRF2 is considered to be implicated in high compensatory expression of TPP1, although the specific mechanism has yet to be revealed.

Hypotheses of the mechanism

On the premise of the telomere damage and repair theory in conjunction with results mentioned above, we put forward hypotheses about IA's etiological mechanism as followed.

The first is about alterations in vascular microenvironment. Under long-term synergistic influences of alterations in mechanical factors such as blood pressure and haemodynamic forces and chemical factors such as serum Hcy and E2 levels, these high risk factors associated with IAs are very prone to significant changes in environments of inflammation, stress, and oxidation.

The second is about truncation of cerebrovascular telomeres. On the one hand, as a result of

activating the mitogen-activated protein kinase (MAPK), these inflammatory factors and oxidative stress factors incur hyper-proliferation of vascular endothelial cells (VECs) and vascular smooth muscle cells (VSMCs) and, further, accelerate the shortening of cerebrovascular telomeres at the mitosis stage. On the other hand, in a bid to hamper recovery of the telomere-telomerase system and promote truncation of telomeres ultimately, these factors both directly inhibit expression and activity of telomerase, and indirectly inhibit activity of telomerase attributable to escalating expression of telomere-binding proteins including TRF2, TPP1, and POT1.

When telomeres are truncated to a certain extent by pathways mentioned above, on the one hand, DNA strands of cerebrovascular endothelial cells and smooth muscle cells accelerate apoptosis on account of loss of telomere safeguards and degradation of cellular functions; on the other hand, shortened telomeres directly active p53 pathways and increase levels of apoptosis. As a large number of VECs are induced into apoptosis, vascular walls gradually lose original forms and become not smooth any more. Cells undergo phase I aneurysm neoplasia during this period and numerous VECs and VSMCs are induced into further apoptosis due to continuous effects of these factors. Cerebrovascular walls consistently become thinner and this thinning process in turn triggers vascular remodelling which alters forms and functions of vascular walls in the meantime. Persistent mechanical effects such as hypertension and haemodynamic forces promote gradual bulging of thinned vascular walls and thus induce phase II aneurysms. At the next stage, with non-stop telomere truncation-induced apoptosis and constant thinning and remodelling of vascular walls, the wall sheer stress (WSS) credited to blood flow vortices through bulgy phase II aneurysms alters. This alteration exacerbates telomere damages and apoptosis. Together with hypertension, thinned vascular walls continue protruding. These increased swellings develop into phase III aneurysms eventually. When vascular walls are thinned to a certain extent, they cannot bear hypertension and haemodynamic forces any more. Consequently, IA ruptures occur before spontaneous subarachnoid haemorrhage (sSAH) is induced, both of which endanger people's health and life (**Figure 5**).

Conclusion and envision

In conclusion, on the basis of the telomere damage and repair theory, the etiological mechanism of IAs is primarily discussed following the study on variations of telomere lengths and expression levels of telomerase and telomere-binding proteins. This research has potential to be the cornerstone of the further study on the formation and rupture of IAs and to provide new access to clinical prevention and therapeutic agents.

Contradictions and limitations

However, there are drawbacks and limitations in this experiment. Due to the contradiction between vascular construction and vascular telomere detection, this experiment fails to provide direct proofs of correlations between IAs and telomeres, especially between disease stages and telomere lengths. In addition, formation of human IAs is rather a complicated and time-consuming process and a synergy of multiple mechanisms, whereas the perspective of this experiment was confined to telomere damage and repair and other regulation mechanisms were excluded. More perspectives hereby are expected to be considered in the next step of research in order to have a thorough understanding of IAs and carry out further studies.

Acknowledgements

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Disclosure of conflict of interest

None.

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