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Morphometric analysis of the human endoneurial extracellular matrix components during aging

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Abstract: The aim of this study was to analyze the expression of extracellular matrix (ECM) proteins in human endoneurium during aging. We harvested 15 cadaveric sural nerves, distributed in 3 age groups (I: 25-44, II: 45-64, III: 65-86 years old). Histological sections were stained immunohistochemically for the presence of collagen type I, type IV and laminin, and the ImageJ processing program was used in morphometrical analysis to determine the percentages of these endoneurial proteins. In two younger groups, the endoneurial matrix of the sural nerve was composed from about equal proportions of these proteins, which may be considered a favorable microenvironment for the regeneration of nerve fibers. Linear regression analysis showed a significant increase in endoneurial collagen type IV with age, while collagen type I and laminin significantly decreased during the aging process. In cases older than 65 years, remodeling of the endoneurial matrix was observed to be significantly higher for the presence of collagen type IV, and lower for the expression of collagen type I and laminin. This age-related imbalance of ECM proteins could represent a disadvantageous microenvironment for nerve fiber regeneration in older adults. Our findings contribute to the development of therapeutic approaches for peripheral nerve regeneration.

Keywords: endoneurium; collagen type IV; collagen type I; laminin; aging

INTRODUCTION

Aging is related to a series of small, microscopic damages that progress to larger cellular damage, leading to cell dysfunction and consequent tissue breakdown [1]. Age-related changes in peripheral nerves are associated with functional disorders caused by a nerve fiber loss, decrease in axon density [2], myelin abnormalities, changes in connective tissue matrix and altered vascularization [3]. The supporting connective tissue that occupies the space between nerve fibers is known as the endoneurium. The endoneurial extracellular matrix (ECM) molecules are important for maintaining appropriate extrinsic conditions for nerve integrity and function, as well as for stimulating axon regeneration after nerve injury [4]. Collagen type I, type III, type IV, fibronectin and laminin are found in the endoneurial ECM of the rat [5]. Collagen fibers lie mostly parallel to the longitudinal axis of the nerve trunk and are particularly located around nerve fibers forming the walls of endoneurial tubes [6]. The most common cell type of the endoneurium is the Schwann cell, which represents about 70% of the endoneurial content that also contains endoneurial fibroblasts, pericytes, endothelial cells and macrophages [7]. The basal lamina of Schwann cells attracts regenerating axons and is considered as another source of ECM, being rich in collagen type IV, laminin, fibronectin and nidogens [8].

Spontaneous axonal regeneration is not an option in an injured or lacerated nerve. There are several surgical techniques in practice with varying outcomes,

such as neurosutures, nerve transfers, autografts and allografts, and nerve guidance conduits [9]. Autologous nerve grafting is the gold standard technique for larger gaps [10] in the peripheral nervous system (PNS), with the sural nerve as a preferred donor nerve [8]. The sural nerve is a sensory nerve that supplies the skin over the lateral and posterior ankle and lateral foot to the base of the fifth toe [11]. When used as a graft, it provides a scaffold with hundreds to thousands of basal lamina tubes to support Schwann cell and axon migration [9]. Although being a pure sensory nerve, it was also shown to be a good cable-grafting donor nerve in motor nerve recovery in a child [12], although in most cases it is used in adults and older patients, which raises the issue of the possible influence of aging on the outcome of axon recovery.

Certain ECM components remodel in senescent cells, and thus, by being involved in the development of age-related diseases [13], they could impair axonal regeneration [14,15]. Recent studies have targeted ECM remodeling as a promising therapeutic approach to wound repair and regeneration [16]. However, there are only a few reports dealing with the morphological changes within the endoneurium of the peripheral nerve associated with aging, and these findings have been obtained in animals [3,4,17-22]. To the best of our knowledge, there are no papers dealing with quantitative age-related changes of the components of the ECM in the endoneurium of the human peripheral nerve.

To determine the differences in expression of the components of the ECM that are involved in nerve fiber regeneration during the aging process in human peripheral nerve, we measured the percentage of collagen type I, type IV and laminin in the endoneurium of the human sural nerve in different age groups.

MATERIALS AND METHODS

Materials

Human sural nerves were obtained during routine autopsies of 15 cadavers (5 males and 10 females) at the Department of Forensic Medicine of the Faculty of Medicine of the University of Niš, Serbia, following the guidelines of the Declaration of Helsinki and the Approval of the Ethics Committee of the Faculty No. 01-9002-8. Sural nerve samples were obtained within 12 h post mortem by dissection of the skin that covers the posterior side of the lateral malleolus. The cadavers were without any previously diagnosed nervous system disorders or any other systemic conditions that could impair nerve tissue. The age of the cadavers ranged from 25 to 86 years. They were classified into 3 age groups as follows: in the 1st group (I), the age ranged from 25 to 44 years (n=5), in the 2nd (II) from 45 to 64 years (n=4), and in the 3rd (III) 65 years and older (n=6).

Immunohistochemistry

Tissue samples were fixed in 10% buffered formalin for 24 h and were routinely processed in paraffin blocks. Tissue sections 5 μm thick were made using a Leica microtome and stained immunohistochemically with antibodies against collagen type I, type IV, and laminin. Sections of the sural nerve stained only with secondary antibody served as the negative control.

The obtained tissue sections were incubated at 60°C for 20 min and subsequently dewaxed in xylene and rehydrated in a descending ethanol series. Antigen retrieval was performed with trypsin for 90 min at 42°C, followed by further incubation in 3% hydrogen peroxide solution for 10 min at room temperature to block the tissue hydrogen peroxide. After washing in tris buffer (pH 7.2), the slides were blocked for 30 min using 10% fetal bovine serum and incubated overnight with primary antibodies (rabbit polyclonal anti-collagen I antibody, Abcam, UK, ab34710, 1:400; rabbit polyclonal anti-Laminin antibody, Abcam, ab11575, 1:25; rabbit polyclonal anti-collagen IV antibody, Abcam, ab6586, 1:400) at 4°C in a humid chamber. The sections were washed in the tris buffer once more and the secondary antibody and streptavidin-HRP were applied each for 30 min at room temperature. As a visualization system, we used a rabbit-specific HRP/DAB detection IHC kit (Abcam, ab64261). Finally, tissue sections were counterstained with hematoxylin, rinsed with tap water, dehydrated in an ascending ethanol series and mounted with Canada balsam.

Morphometric analysis

Histological slices were analyzed by light microscopy under different magnifications. For all examined

antibodies, 10 fields containing the endoneurium of each sural nerve were randomly selected and captured with a digital camera (1.3 megapixels) under 100× lens magnification. The obtained 24-bit RGB images were then processed and analyzed with ImageJ software (http://rsb.info.nih.gov/ij/). Analysis of the endoneurial ECM components was performed by thresholding the image according to the following procedure: each original image was duplicated using the option Image/Duplicate; the plug-in Image/Adjust/Color Threshold was activated wherein the black and white (B&W) option was checked as the Threshold color. Using polygonal selection from the menu, at least 5 positive pixels (brown pixels) were chosen from the duplicated image. Using the option Sample in the Threshold Color window, only positive pixels were retained on the image (black pixels). The obtained image was compared to the original one and if the image processing was satisfactory, the duplicated images were converted to binary ones using the option Process/Binary/Make Binary. In the program menu, the option Area Fraction was checked using the option Analyze/Set measurements. After conversion of the analyzed image into the binary one using the option Analyze/Measure, we obtained the result that represented the percentage (%) of positive pixels within the analyzed area (image). Thereby we acquired the percentage (%) values of collagen type IV (P_{CIV}), collagen type I (P_{CI}) and laminin (P_L) in each examined field. The collagen type IV, type I, and laminin percentage contents of the endoneurium for a single case were obtained as the mean value of its 10 analyzed fields of vision. A similar method for the quantification of the endoneurial ECM components on digital images was previously applied [23].

Statistical analysis

Statistical analysis was performed using the SPSS (version 16) statistical package. Linear regression analysis was conducted to evaluate the correlation between age as predictor and the obtained morphometric parameters (endoneurial percentages of collagen type IV, type I, and laminin, respectively) as the outcome variables. The significance of differences between the mean values of the morphometric parameters of the established age groups was analyzed by one-way ANOVA and additionally by the Tukey-Kramer post-hoc test.

RESULTS

Histological analysis of immunoreactivity

Collagen type IV immunopositivity was observed in all nerve connective tissue sheaths (Fig. 1). It was weak in the epineurium and mostly located in the basement membranes of blood vessels. The perineurium showed a laminate pattern of the positive reaction on the same antibody (Fig. 1A, D). In the endoneurium a positive reaction was the most frequently observed around the nerve fibers and in the walls of blood vessels (Fig. 1A, D, G).

The immunohistochemical reaction in the 1st age group was weaker and in the form of fine circular immunopositivity around the nerve fibers (Fig. 1B). This was more emphasized around the smaller nerve fibers of the 2nd age group (Fig. 1E). In the 3rd age group, the same form of stronger immunoreactivity was detected around both smaller and larger nerve fibers (Fig. 1H).

The immunohistochemical reaction on collagen type I was more intense in the epineurial and endoneurial sheaths (Fig. 2). In the endoneurium, reactivity was diffuse and much stronger in younger

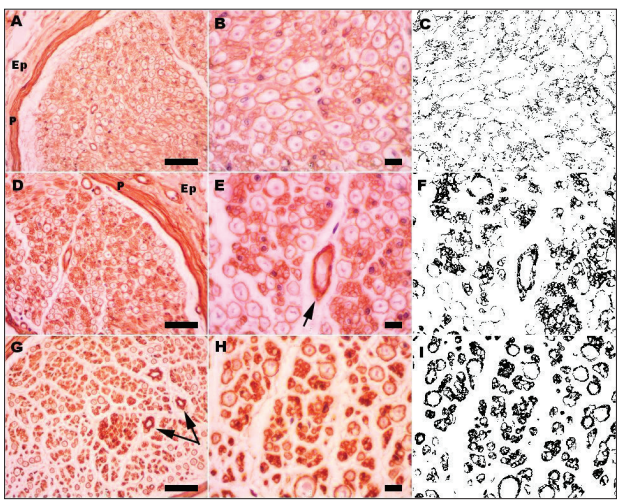

Fig 1. Immunoreactivity of collagen type IV. Cross-section of the sural nerve from a 31-year-old cadaver, **A** – scale bar=50 μm, **B** – scale bar=10 μm, **C** – binary image obtained after processing image 1B. Cross-section of the sural nerve from a 51-year-old cadaver, **D** – scale bar=50 μm, **E** – scale bar=10 μm, **F** – binary image after processing image 1E. Cross-section of the sural nerve from a 75-year-old cadaver, **G** – scale bar=50 μm, **H** – scale bar=10 μm, **I** – binary image after processing image 1H. Ep – epineurium; P – perineurium; arrows show positive immunoreactivity of collagen type IV in the wall of the blood vessel (internal positive control).

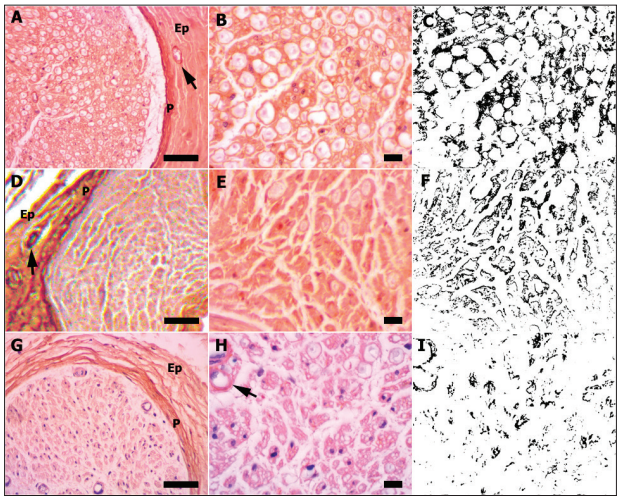

Fig. 2. Immunoreactivity of collagen type I. Cross-section of the sural nerve from a 36-year-old cadaver, **A** – scale bar=50 μm, **B** – scale bar=10 μm, **C** – binary image obtained after processing image 2B. Cross-section of the sural nerve from a 64-year-old cadaver, **D** – scale bar=50 μm, **E** – scale bar=10 μm, **F** – binary image after processing image 2E. Cross-section of the sural nerve from an 81-year-old cadaver, **G** – scale bar=50 μm, **H** – scale bar=10 μm, **I** – binary image after processing image 2H. Ep – epineurium; P – perineurium; arrows show positive immunoreactivity of collagen type I in the wall of the blood vessel (internal positive control).

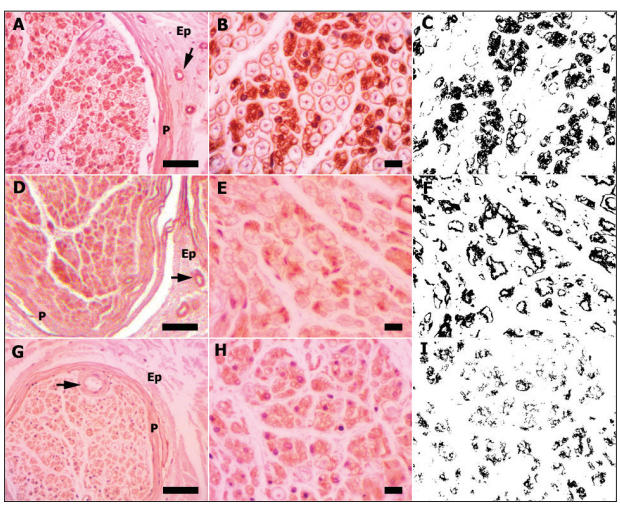

Fig. 3. Immunoreactivity of laminin. Cross-section of the sural nerve from a 31-year-old cadaver, **A** – scale bar=50 μm, **B** – scale bar=10 μm, **C** – binary image obtained after processing image 3B. Cross-section of the sural nerve from a 58-year-old cadaver, **D** – scale bar=50 μm, **E** – scale bar=10 μm, **F** – binary image after processing image 3E. Cross-section of the sural nerve from a 75-year-old cadaver, **G** – scale bar=50 μm, **H** – scale bar = 10 μm, **I** – binary image obtained after processing image 3H. Ep – epineurium; P – perineurium; arrows show positive immunoreactivity of laminin in the wall of the blood vessel (internal positive control).

cases (Fig. 2B,E). In older cases, significantly weaker immunoreactivity of collagen type I was observed in the endoneurium (Fig. 2H).

The immunohistochemical reaction on laminin was weaker in the epineurium and mostly present in the basement membranes of blood vessels. In the perineurium, it appeared as laminated (Fig. 3). However, in contrast to collagen type IV, laminin exhibited a stronger positive reaction in younger cases, particularly around the small nerve fibers (Fig. 3B). Immunoreactivity of the endoneurium was much weaker in the older age groups and localized around nerve fibers (Fig. 3E, H).

Morphometric analysis and correlation between age and endoneurial percentages

The results of morphometric analysis are presented in Table 1. Linear regression analysis showed that there was a significant increase in the mean endoneurial collagen type IV percentage with age ($F(1,13)=28.44$, $P<0.001$) (Table 1). The mean endoneurial percentage of collagen type I ($F(1,13)=10.45$, $P=0.007$) (Table 1), as well as of laminin ($F(1,13)=12.39$, $P=0.004$) significantly decreased during the aging process (Table 1). These relationships were identified with the following 3 equations:

Table 1. Results of the morphometric analysis for all 15 analyzed cases.

Case	Age	Group	Gender	P_{CIV} (%)	P_{CI} (%)	P_L (%)
1	25	I	male	10.57	10.78	13.45
2	31	I	female	13.97	14.87	20.67
3	36	I	male	12.28	17.06	17.94
4	39	I	female	13.81	18.77	20.87
5	43	I	male	10.39	20.44	11.70
6	51	II	female	18.12	14.30	11.74
7	55	II	male	14.46	12.36	9.73
8	58	II	female	17.31	20.73	16.73
9	64	II	male	11.36	9.54	13.79
10	67	III	female	16.26	9.06	16.09
11	73	III	female	17.73	8.14	8.26
12	75	III	female	20.16	4.83	7.59
13	80	III	female	22.30	11.64	9.46
14	81	III	female	21.28	3.58	10.80
15	86	III	female	23.26	6.41	6.91

P_{CIV} – Endoneurial percentage of collagen type IV
P_{CI} – Endoneurial percentage of collagen type I
P_L – Endoneurial percentage of laminin

Table 2. Mean values of the age, collagen type I, collagen type IV and laminin endoneurial percentages of the analyzed age groups.

Parameter	Group	N	Mean	SD	Upper limit 95% CI (%)	Lower limit 95% CI (%)
Age	I	5	34.8	7.014	26.09	43.51
	II	4	57	5.477	48.28	65.72
	III	6	77	6.723	69.94	84.06
P_{CIV} (%)	I	5	12.20	1.71	10.08	14.32
	II	4	15.31	3.07	10.43	20.19
	III	6	20.17	2.70	17.33	23.00
P_{CI} (%)	I	5	16.38	3.75	11.73	21.04
	II	4	14.23	4.75	6.67	21.79
	III	6	7.28	2.95	4.19	10.37
P_{L} (%)	I	5	16.93	4.18	11.73	22.12
	II	4	13.00	2.99	8.24	17.76
	III	6	9.85	3.35	6.33	13.37

CI – Confidence interval
P_{CIV} – Endoneurial percentage of collagen type IV
P_{CI} – Endoneurial percentage of collagen type I
P_{L} – Endoneurial percentage of laminin

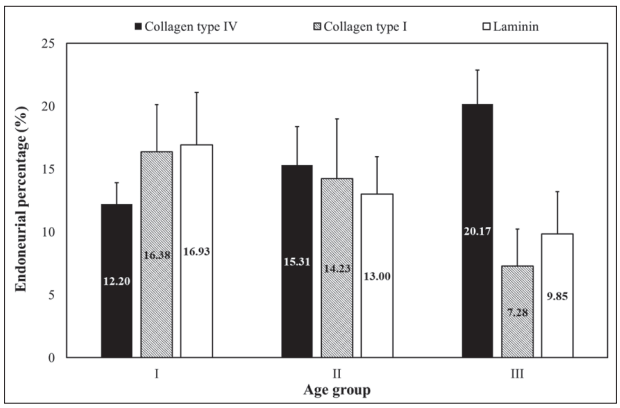

Fig. 4. Mean values of the collagen type IV, collagen type I, and laminin endoneurial percentages of the analyzed age groups.

Collagen type IV percentage = 0.18 x age + 5.85 (r=0.83);

Collagen type I percentage = 22.88 – 0.19 x age (r=0.70);

Laminin percentage = 22.39 – 0.16 x age (r=0.67).

A more detailed analysis of the morphometric parameter dynamics with age was performed with one-way ANOVA. The mean endoneurial percentage of collagen type IV ($F(2,12)=13.92$, $P=0.001$), collagen type I ($F(2,12)=8.94$, $P=0.004$) and of laminin ($F(2,12)=5.35$, $P=0.022$) were significantly different between the evaluated age groups (Table 2, Fig. 4). Tukey's post-hoc test additionally showed that in the case of collagen type IV, the mean endoneurial percentage in group III was significantly higher than in groups II and I ($P<0.05$). The mean endoneurial percentage of group II was higher than for group I, but this difference was not significant ($P>0.05$). In contrast to collagen type IV, the mean endoneurial percentage of collagen type I of the 3rd age group was significantly lower than that of the 1st and 2nd age groups ($P<0.05$). The mean endoneurial percentage of the 2nd age group was insignificantly lower than the mean endoneurial percentage of the 1st age group ($P>0.05$). Finally, for the mean laminin endoneurial percentage, Tukey's post-hoc test showed that its value in the 1st age group was significantly higher in relation to the 3rd ($P<0.05$), and insignificantly ($P>0.05$) higher than in the 2nd age group. The mean endoneurial percentage of the 2nd age group was insignificantly higher than the mean endoneurial percentage of the 3rd age group ($P>0.05$).

DISCUSSION

Collagens play an important role in PNS development (axonal guidance, synaptogenesis, terminal differentiation of myelination of Schwann cells, establishment of the normal architecture of the nervous system), as well as in several pathophysiological processes [24]. Collagens in the PNS are produced by both Schwann cells and fibroblasts [18,25]. The types of collagens that are present in the PNS can be classified into fibrillar (collagen types I, III, and V) and basement membrane (type IV) collagens [25].

We observed that the ECM content of the endoneurium was characterized by the presence of laminin and collagen types I and IV. Our results suggest that the quantity and immunohistochemical reaction of collagen type IV around the nerve fibers increases with aging, being the most intense in the oldest group, while the quantity of collagen type I decreases and is seen only occasionally in the endoneurium of the oldest group. Laminin immunopositivity was observed in a form of a ring-like structure surrounding each nerve fiber, with a tendency to decrease in quantity during aging. It is well known that aging influences the increase in quantity of collagen type IV in the basement membranes of many organs, such as the brain, eye blood vessels, renal corpuscles and internal ear basement membranes [26-29]. On the other hand, the decrease in collagen

types IV and I in the endoneurium has been reported in the rat oculomotor nerve during aging [18]. However, these results were obtained by examining the motor nerve, and our material was taken from the human cadaveric sensory nerve. Another animal study did not find quantitative changes in the collagen content of the endoneurium in the sciatic nerve during aging of Wistar rats [20], although the authors determined the total amount of collagen without taking into consideration specific types. The decrease in the amount of laminin during aging could be connected with the age-induced decrease in the number of Schwann cells around myelinated axons [3].

ECM is considered to be a static structure that provides support, separation and a filtering function in tissues, and after detailed studies of interactions between ECM proteins (collagens, laminin, nidogen, tenascin, proteoglycans) and cellular membrane receptors, a broader range of functions has been suggested [25]. Interactions in the ECM can activate intercellular signals and control cell processes, such as migration, differentiation and survival [30]. Developing axons elongate in an extracellular substrate that supports their growth. Collagen type I is generally considered such a substrate [24]. In our study we detected a significant increase in collagen type IV, which was accompanied by a significant decrease in immunoreactivity of collagen type I and laminin in the endoneurium of the sural nerve during the aging process. Differences between the examined components of the ECM were not higher than 5% in age groups I and II (as the percentages of collagen type I and laminin are almost identical). However, after the age of 65, an imbalance between these components of the ECM increased and the amount of collagen type IV was more than 2-fold larger than of collagen type I and laminin.

The content of a specific collagen type is tissue- and time-specific. Collagen type IV is produced by Schwann cells as a component of the basement membrane required for tissue repair, survival and regular tissue functioning, while collagen type I, being a product of fibroblasts, is a glue-like substance, meant to preserve the structure of the tissue and to fill the endoneurial space [31]. Replacing the basement membrane matrix by a dense interstitial matrix can lead to the loss of specialized function of that ECM and consequently to organ dysfunction [32]. In liver fibrogenesis, an increase in the amount of collagen type IV is observed; however, it is the increased ratio of collagen type I to collagen type IV that leads to dysfunction [31]. Our results indicate that aging may cause a disbalance in the ratio of collagen type I to collagen type IV in the endoneurium due to elevated collagen type IV and degradation of collagen type I. It was shown that the turnover of these proteins is differently regulated during aging, as in young rats a more than 100-fold higher turnover of interstitial collagen type I as compared to older animals was reported, while the amount of basement membrane collagen type IV was 3.5-fold higher in older rats [33]. The production of collagen type I is generally considered to progressively decrease with age, along with increased degradation either from increased activity of collagenase and matrix metalloproteinases (MMPs), or from a decrease in tissue inhibitors of MMPs [34].

Laminin, as a basement membrane protein, is expected to show a similar degree of turnover of collagen type IV in different tissues, as reported in the literature [35]. However, our data are in accordance with the results obtained from age-related degenerated human cartilage tissues [36] and age-dependent changes in the human inner limiting membrane of the eye [37], which showed a decrease or absence in laminin deposition that was followed by an increased presence of collagen type IV in the basement membrane. A study on nerve grafting between younger and older rats showed a 50-110% higher level of laminin polypeptides in the former, which was functionally followed by improved axon sprouting after the grafting of a younger donor to an older recipient than vice versa. Therefore, it was suggested that age-induced changes of the ECM components in the PNS probably play an important role in age-related impaired sprouting of sensory axons in nerve grafts [38].

CONCLUSIONS

The findings of this study showed that the ratio of investigated ECM components of the endoneurium of the sural nerve significantly changed during the aging process and that after the age of 65 a significant remodeling of the endoneurial matrix occurs. Thereafter, the aging endoneurium contains a significantly higher percentage of collagen type IV and lower percentages

of collagen type I and laminin. This finding suggests that peripheral nerves are less resistant to age-related collagen type I and laminin loss, but also that the endoneurial microenvironment during aging is unfavorable for nerve fiber regeneration. The change in balance of ECM proteins in the endoneurium during different life stages might be of importance in understanding the process of axon regeneration and should be taken into account for enhancing current and developing novel therapeutic strategies for peripheral nerve injury.

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Conflict of interest disclosure: The authors declare that they have no conflict of interest.

REFERENCES

1. Bhatia-Dey N, Kanherkar RR, Stair SE, Makarev EO, Csoka AB. Cellular Senescence as the Causal Nexus of Aging. Front Genet. 2016;7:13.
2. Goto K, Naito K, Nakamura S, Nagura N, Sugiyama Y, Obata H, Kaneko A, Kaneko K. Protective mechanism against age-associated changes in the peripheral nerves. Life Sci. 2020; 253:117744.
3. Ceballos D, Cuadras J, Verdú E, Navarro X. Morphometric and ultrastructural changes with ageing in mouse peripheral nerve. J Anat. 1999;195(4):563-76.
4. Dubovy P, Klusáková I, Svíženská I. A quantitative immunohistochemical study of the endoneurium in the rat dorsal and ventral spinal roots. Histochem Cell Biol. 2002;117:473-80.
5. Lorimier P, Mezin P, Labat Moleur F, Pinel N, Peyrol S, Stoebner P. Ultrastructural localization of the major components of the extracellular matrix in normal rat nerve. J Histochem Cytochem. 1992;40:859-68.
6. Geuna S, Raimondo S, Ronchi G, Di Scipio F, Tos P, Czaja K, Fornaro M. Chapter 3: Histology of the peripheral nerve and changes occurring during nerve regeneration. Int Rev Neurobiol. 2009;87:27-42.
7. Stierli S, Napoli I, White IJ, Cattin AL, Monteza Cabrejos A, Garcia Calavia N, Malong L, Ribeiro S, Nihouarn J, Williams R, Young KM, Richardsin WD, Lloyd AC. The regulation of the homeostasis and regeneration of peripheral nerve is distinct from the CNS and independent of a stem cell population. Development. 2018;145:dev170316
8. Gonzalez-Perez F, Udina E, Navarro X. Extracellular Matrix Components in Peripheral Nerve Regeneration. Int Rev Neurobiol. 2013;108:257-75.
9. Houschyar KS, Momeni A, Pyles MN, Cha JY, Maan ZN, Duscher D, Jew OS, Siemers F, van Schoonhoven J. The Role of Current Techniques and Concepts in Peripheral Nerve Repair. Plast Surg Int. 2016;2016:4175293.
10. Carvalho CR, Reis RL, Oliveira JM. Fundamentals and Current Strategies for Peripheral Nerve Repair and Regeneration. Adv Exp Med Biol. 2020;1249:173-201.
11. Bilbao J, Schmidt RE. Normal Anatomy of the Peripheral (Sural) Nerve. In: Bilbao J, Schmidt RE, editors. Biopsy diagnosis of peripheral neuropathy. 2nd ed. Springer; 2015. p. 21-41.
12. Pillutla P, Nix E, Elberson BW, Nagy L. Complete Femoral Nerve Transection with Sural Nerve Cable Graft in a 21-Month-Old Child. J Neurosci Rural Pract. 2019;10(1):139-41.
13. Amaya-Montoya M, Pérez-Londoño A, Guatibonza-García V, Vargas-Villanueva A, Mendivil CO. Cellular Senescence as a Therapeutic Target for Age-Related Diseases: A Review. Adv Ther. 2020;37(4):1407-24.
14. Kovacic U, Sketelj J, Bajrović FF. Chapter 26: Age-related differences in the reinnervation after peripheral nerve injury. Int Rev Neurobiol. 2009;87:465-82.
15. Verdú E, Ceballos D, Vilches JJ, Navarro X. Influence of aging on peripheral nerve function and regeneration. J Peripher Nerv Syst. 2000;5(4):191-208.
16. Bonnans C, Chou J, Werb Z. Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol. 2014;15(12):786-801.
17. Jeronimo A, Jeronimo CAD, Filho OAR, Sanada LS, Fazan VPS. Microscopic anatomy of the sural nerve in the postnatal developing rat: a longitudinal and lateral symmetry study. J Anat. 2005;206:93-9.
18. Bahcelioglu M, Elmas C, Kurkcuoglu A, Calguner E, Erdogan D, Kadioglu D, Gözil R. Age-related immunohistochemical and ultrastructural changes in rat oculomotor nerve. Anat Histol Embryol 2008;37(4):279-84.
19. Goss JR, Stolz DB, Robinson AR, Zhang M, Arbujas N, Robbins PD, Glorioso JC, Niedernhofer LJ. Premature Aging-related Peripheral Neuropathy in a Mouse Model of Progeria. Mech Ageing Dev. 2011;132(8-9):437-42.
20. Esquisatto MAM, de Aro AA, Fêo HB, Gomes L. Changes in the connective tissue sheath of Wistar rat nerve with aging. Ann Anat. 2014;196:441-8.
21. Yuan X, Klein D, Kerscher S, West BL, Weis J, Katona I, Martini R. Macrophage Depletion Ameliorates Peripheral Neuropathy in Aging Mice. J Neurosci. 2018;38(19):4610-20.
22. Biasibetti E, Bisanzio D, Mioletti S, Amedeo S, Iuliano A, Bianco P, Capucchio MT. Spontaneous Age-related Changes of Peripheral Nerves in Cattle: Morphological and Biochemical Studies. Anat Histol Embryol. 2016;45(2):100-8.

23. Hill R. Extracellular matrix remodelling in human diabetic neuropathy. J Anat 2009; 214(2):219-25.
24. Hubert T, Grimal S, Carroll P, Fichard-Carroll A. Collagens in the developing and diseased nervous system. Cell Mol Life Sci 2009;66(7):1223-38.
25. Koopmans G, Hasse B, Sinis N. Chapter 19: The role of collagen in peripheral nerve repair. Int Rev Neurobiol 2009; 87:363-79.
26. Uspenskaia O, Liebetrau M, Herms J, Danek A, Hamann GF. Aging is associated with increased collagen type IV accumulation in the basal lamina of human cerebral microvessels. BMC Neurosci. 2004;5:37.
27. Chen L, Miyamura N, Ninomiya Y, Handa JT. Distribution of the collagen IV isoforms in human Bruch's membrane. Br J Ophthalmol. 2003;87(2):212-5.
28. Ishiyama A, Mowry SE, Lopez IA, Ishiyama G. Immunohistochemical distribution of basement membrane proteins in the human inner ear from older subjects. Hear Res. 2009;254(1-2):1-14.
29. Anderson S, Brenner BM. Effect of aging on the renal glomerulus. Am J Med. 1986;80:435-44.
30. Aszódi A, Legate KR, Nakchbandi I, Fässler R. What mouse mutants teach us about extracellular matrix function. Annu Rev Cell Dev Biol. 2006;22:591-621.
31. Karsdal MA, Nielsen SH, Leeming DJ, Langholm LL, Nielsen MJ, Manon-Jensen T, Siebuhr A, Gudmann NS, Rønnow S, Sand JM, Daniels SJ, Mortensen JH, Schuppan D. The good and the bad collagens of fibrosis – their role in signaling and organ function. Adv Drug Deliv Rev. 2017;121:43-56.
32. Genovese F, Manresa AA, Leeming DJ, Karsdal MA, Boor P. The extracellular matrix in the kidney: a source of novel non-invasive biomarkers of kidney fibrosis? Fibrogenesis Tissue Repair. 2014;7(1):4.
33. Karsdal MA, Genovese F, Madsen EA, Manon-Jensen T, Schuppan D. Collagen and tissue turnover as a function of age: Implications for fibrosis. J Hepatol. 2016;64(1):103-9.
34. Sprenger CC, Plymate SR, Reed MJ. Aging-related alterations in the extracellular matrix modulate the microenvironment and influence tumor progression. Int J Cancer. 2010;127(12):2739-48.
35. Keeley DP, Hastie E, Jayadev R, Kelley LC, Chi Q, Payne SG, Jeger JL, Hoffman BD, Sherwood DR. Comprehensive Endogenous Tagging of Basement Membrane Components Reveals Dynamic Movement within the Matrix Scaffolding. Dev Cell. 2020;54(1):60-74.e7.
36. Foldager CB, Toh WS, Gomoll AH, Olsen BR, Spector M. Distribution of Basement Membrane Molecules, Laminin and Collagen Type IV, in Normal and Degenerated Cartilage Tissues. Cartilage. 2014;5(2):123-32.
37. Candiello J, Cole GJ, Halfter W. Age-dependent changes in the structure, composition and biophysical properties of a human basement membrane. Matrix Biol. 2010;29(5):402-10.
38. Kovačič U, Žele T, Marš T, Sketelj J, Bajrović FF. Aging impairs collateral sprouting of nociceptive axons in the rat. Neurobiol Aging. 2010;31(2):339-50.