Journal of the Anatomical Society of India 66 (2017) 1–6

Contents lists available at ScienceDirect

Journal of the Anatomical Society of India

journal homepage: www.elsevier.com/locate/jasi

Original article

Activation of Akt and the signaling of phosphorylated Akt in the L5 dorsal root ganglia in aging rats

Hartini Md Yon*, Murali Naidu

Department of Anatomy, Faculty of Medicine, University Malaya, 50603 Kuala Lumpur, Malaysia

ARTICLE INFO

Article history:
Received 8 February 2016
Accepted 25 May 2017
Available online 30 May 2017

Keywords:
Aging
Akt
Dorsal root ganglia
Satellite glial cell

ABSTRACT

Introduction: Protein kinase B (PKB)/Akt is a kinase that is responsive towards insulin-like growth factor (IGF-1) and nerve growth factor (NGF). Detected in the cytosol, Akt is activated by phosphorylation at Thr308/Ser473 residue. The phosphorylated Akt (pAkt) is translocated into the nucleus via the of phosphatidylinositide 3-kinases (PI3K)-Akt pathway. Contradicting results have been reported on the expression of Akt and pAkt in aged animals. The study is conducted to determine the effect of aging on the Akt and pAkt signaling in the L5 dorsal root ganglia (DRG).
Methods: Twenty-four female *Sprague-dawley* rats were sacrificed at 3, 10, 20 and 27 months. L5 DRG was harvested and subjected to immunofluorescence staining.
Results: The expression of Akt and pAkt was detected in almost all neurons in all groups. Image analyzer analysis showed that the average intensity of Akt signaling increased from M10 to M27 compared to the M3. However, the average intensity of pAkt signaling was only significantly increased in M27 compared to the younger animals.
Conclusion: The expression of Akt and pAkt may indicate that increase activity is taking place in the aged neurons. The activation of Akt and its phosphorylation may be one of the mechanisms in response towards aging process.

1. Introduction

The process of aging has been known to affect peripheral nervous function. For instance, the deterioration of somatosensory function is known to occur and is extensively investigated in aged subjects.[1–3] Meanwhile, the loss of motor function in aging has been identified as the main cause of impairment of physical activity and independence in the elderly.[4–7] The alteration of physiological function in aged individual is associated with, among others, the degeneration of cutaneous receptors,[2] morphological changes of peripheral nerve,[5,8] as well as changes in axonal components,[4,9] slowing of motor response towards stimuli [6] and alteration in neuropeptides levels and their receptors.[10,11]

Protein kinase B, PKB/Akt, is a kinase responsive towards insulin-like growth factor and nerve growth factor that controls metabolism, growth, apoptosis and differentiation.[12] Akt is phosphorylated at Thr 308 and Ser 473 residue to generate high level activity.[13] Three isoforms of Akt (Akt1, Akt2 and Akt3) have been found in mammalian cells,[14–16] normally in the cytosol in its inactive form and can be activated by the stimulation of numerous transmembrane receptors.[17] Once activated, the Akt will be phosphorylated and translocated into the nucleus. This requires the activation of phosphatidylinositide 3-kinases, PI3K.[18]

In several studies, Akt activation and phosphorylation is found to be beneficial for the proliferation, protection and survival of cells against neurodegeneration in injury and aging. The expression of Akt and phosphorylated Akt is up-regulated in response towards injury.[19–22] Meanwhile, in aging studies, contradicting results have been reported on the expression of Akt and its phosphorylated form.[23–27] In this study, we explored the effect of aging on Akt signaling and the phosphorylation of Akt in L5 DRG using immunofluorescence double staining procedure. The signaling intensity of each protein was measured at different stages of life. We showed that the intensity of Akt and phosphorylated Akt signaling inL5 DRG is increased at advanced age. Furthermore, at advanced age, the phosphorylated Akt is shown to be translocated into the nucleus of the neurons. Therefore, at this age, the Akt signaling and the phosphorylation of Akt is required, probably to maintain the survival of cells against aging-related neurodegeneration.

* Corresponding author.
E-mail addresses: hartini_mdyon@yahoo.com (H.M. Yon), Murali_naidu@um.edu.my (M. Naidu).

http://dx.doi.org/10.1016/j.jasi.2017.05.009
0003-2778/

2. Materials and methods

All animal procedures were conducted according to the local guidelines for care and use of experimental animals and approved by Institutional Animal Care and Use Committee (IACUC), University of Malaya. Female *Sprague-dawley* rats were used in this study. Animals were obtained from the Animal Experimental Unit, Faculty of Medicine, University of Malaya. All animals were housed in an individual flat surface cage under controlled environment; with a temperature range between 22°–25 °C and 12 h light/12 h darkness. Animals were given free access of standard rat chow and water *ad libitum*. The animals were divided into 4 groups: 3 months (young adult), 10 months (mature), as well as 20 months (early aged) and 27 months (advanced aged) with 6 animals per group.

The animals were weighed prior to the experiment, after which they were anaesthetized with intraperitoneal injection of 3.5% chloral hydrate 1 ml/100 mg body weight and perfused with 4% paraformaldehyde in 0.1 M phosphate buffer solution at pH 7.4. Immediately after perfusion, a cut was made using surgical scissors parallel to the spine to separate the vertebral column from the rib cage. Caudally, the column was cut at the level of hip joint whereas rostrally, the column was cut the level of T8. Muscles attached along the column were removed as much as possible to minimize tissue mass. The vertebral column was then immersed in the fixative for at least 72 h. The spinal cord and the intact DRG were carefully harvested for identification. The lumbar and sacral spinal were identified with the aid of the adherent ventral roots.[28] The tissues were processed, embedded in paraffin wax (Paraplast, USA) and oriented accordingly.

Specimens were sectioned at 5 μm and mounted on poly-L-lysine (Sigma) coated slides and air-dried overnight. Sections were dewaxed, rehydrated and subjected for antigen retrieval treatment in 60 mM sodium citrate buffer pH 6.0. Sections were incubated with 10% sheep normal serum for 20 min to block non-specific binding of immunoglobulin. Then, sections were immediately covered with a mixture of primary antibody; Neurofilament NF200 (mouse monoclonal anti-NF200, 1:200; Sigma) and Akt (rabbit polyclonal anti-Akt, 1:200; Cell Signaling) or phosphorylated Akt Thr308 (rabbit polyclonal anti-phosphorylated Akt; 1:100; Millipore) for 1 h in humidity chamber at room temperature. Subsequently, the sections were treated with a mixture of fluorescent conjugated secondary antibody sheep anti-rabbit CY3 (1:150; Sigma) and sheep anti-mouse FITC (1:150; Sigma) and incubated for 1 h at room temperature. After washing, the slides were mounted using DAPI ProLong Gold Antifade Reagent (Invitrogen). The slides were examined with digital microscope Eclipse 80i (Nikon Instruments) fluorescence microscope. Meanwhile, images were captured and intensities were measured using NIS Image Analyzer (Nikon Instruments).

The means of all parameters were calculated and shown with standard error of mean (SEM). Statistical analysis was performed using one-way ANOVA on all parameters and Tukey or Games-Howell for post-hoc test. Statistical significance was established as $p < 0.05$. All statistical analysis was performed using IBM SPSS Statistic 22 software.

Table 1
Changes in the body weight in rats at various ages. Body weight was measured prior to sacrifice. The body weight increased from M3 to M10. From M10 onwards, the body weight is maintained throughout the study. Values are mean ± SEM; n = 6.

Age of Animals	BW (gm)
M3	208.33 ± 4.01
M10	266.00 ± 10.30*
M20	253.83 ± 12.65*
M27	283.00 ± 14.52*

* Significantly different from M3; $p < 0.05$.

3. Results

The general health of all animals appeared to be intact. There was no apparent weakness of the extremities shown by the animals. Body weight significantly increased from M3 to M10. Minimal mean body weight difference was seen afterwards, the body weight of the animals was maintained until the latest age studied, M27, agreeing with previous studies.[26,29,30] Table 1 represents the summary of body weight of all the groups studied.

Fig. 1 shows the expression of Akt within the L5 DRG at M3, M20 and M27 against NF200, generally known as a marker for large-sized neurons. There was a signaling of Akt in the cytoplasm of both small and large neurons in all groups of animals at various intensities. However, the Akt signaling in the small-sized neurons were more intense compared to the larger-sized, NF-immunoreactive (NF-IR) neurons with some intense signaling on the certain areas in the NF-IR neurons. There was also some but hardly noticeable signaling in the nucleus of the majority of the neurons. It was not possible to determine if the signaling in the cytoplasm is increasing or decreasing as the animals aged. For that reason, we measured the average intensity of the signaling of the tissue using the NIS-Elements Image Analyzer (Fig. 2). The average intensity of NF200 was slightly increased in M10 (23.84 ± 0.92) and M20 (26.11 ± 1.85) compared to M3 (21.41 ± 1.01). The intensity was then decreased at M27 (23.82 ± 1.52). However, the differences were not statistically significant. Hence, the maintained intensity level of NF200 was used as an internal control when measuring the Akt and pAkt intensity.

The measurement of Akt signaling intensity is summarized in Fig. 3. The current study shows that the result for the analysis of variance of Akt signaling intensity was significant. The average intensity of Akt signaling was lowest at M3 (9.23 ± 0.39) and increased as the animals aged. The increase of average intensity at M10 (13.70 ± 1.63) was not significant when compared to M3. The slight increase of average intensity at M20 (14.03 ± 0.60) was not significant when compared to M10 but the difference was significant when compared to M3. In all animals studied, M27 (22.77 ± 1.52) produced the highest average intensity of Akt that was statistically significant when compared to the younger groups.

The activation of Akt/PKB is shown by the phosphorylation of the protein. In this study, we examined the signaling of phosphorylated Akt at the residue of Thr308. At M3, the expression of pAkt was intense in cytoplasm of smaller neurons compared to the larger ones (Fig. 4). Similarly, the signaling of pAkt was seen to be prominent in the cytoplasm of small-sized neurons compared to NF-IR neurons in both M20. In addition, there were noticeable expressions of pAkt in the nucleus of some of the neurons. The expression of pAkt was also intense in the peripheral part of the NF-IR cells compared to the overall intensity of the cells. In M27, the expression of pAkt was robust in most of the nucleus of both small- and large-sized neurons. The signaling in both nucleus and the peripheral area were also intense compared to those seen in the cytoplasm.

The analysis of variance conducted on the average intensity of pAkt signaling demonstrated significant results. The average intensity of pAkt remained constant from M3 to M20. However, the average intensity increased tremendously at M27. The average pAkt intensity per cells is remarkably high (22.17 ± 1.26) compared to the younger groups. The average intensities of pAkt at M27 were significantly different when compared to M3 (6.18 ± 0.61), M10 (11.52 ± 0.56) and M20 (11.15 ± 0.43). Fig. 5 shows the summary of the average of pAkt signaling intensity at different ages studied.

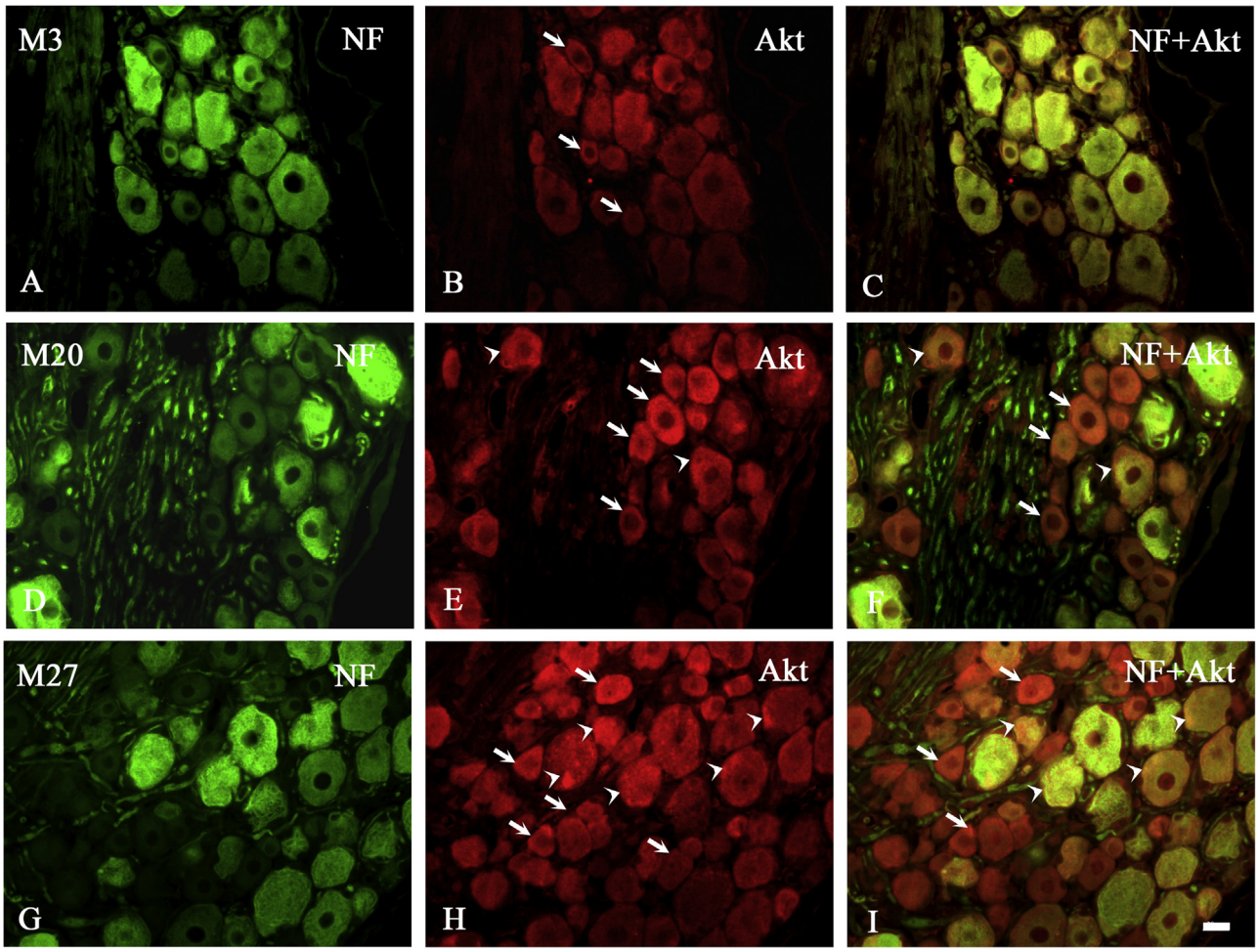

Fig. 1. Akt immune-reactive was detected in almost all DRG neurons, especially in small-sized, non-NF-immunoreactive neurons. A–I: showed the results of double immunofluorescence staining in L5 DRG between Akt (CY3 red; B, E, H) and NF (FITC Green; A, D, G) at the age of M3 (A–C), M20 (D–F) and M27 (G–I). C, F and I show images merged for NF and Akt. The results indicated that Akt is localized in the cytoplasm of majority of cell neurons. The signaling is more prominent in older rats. Arrow – Small-sized neurons, arrowhead – intense Akt staining in the NF-immunoreactive neurons, scale bar A-I: 20 μm.

4. Discussion

Akt is one of the most multifaceted kinases, whose actions may regulate diverse cellular functions; cell survival, cell growth, proliferation, transcription and metabolism.[12] In mice embryo, for instance, Akt is required for normal muscle development. The elimination of Akt1 will prevent muscle differentiation whereas Akt2 deficiency will hinder the maturation of myotubes.[31] However, injured nerve in neonates fails to utilize the survival role of Akt. Instead, Akt in the motoneuron of injured nerve is down-regulated. The decreasing level of Akt will eventually induce neuronal cell death.[20] Another study showed that hyperactivation of Akt in human fibroblast will rapidly induce aging process via mTORC1 activity.[32] In the current study, the signaling of both Akt and phosphorylated Akt were seen in all ages. It is typical for naive cells to have a signaling of Akt and phosphorylated Akt,[19,22,33,34] suggesting that Akt and its phosphorylated form is indeed required for regular cell homeostasis. Signaling of both forms of Akt will increase depending on the metabolic need of the cells.[33] The activation of Akt is a critical event for cell survival.[19,35] Akt becomes activated as a result of phosphorylation at Thr308 and Ser473 residues [13] after its recruitment to plasma membrane.[18]

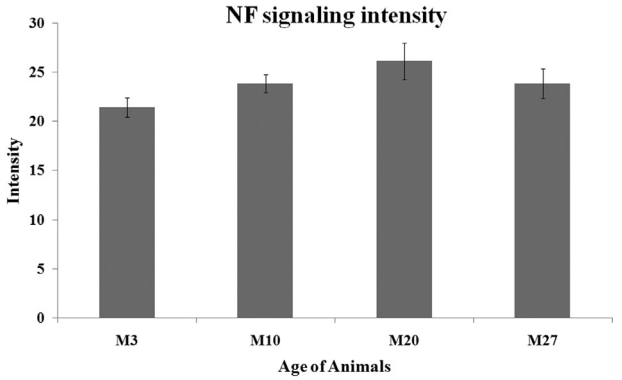

Fig. 2. The signaling of neurofilament-200 (NF) in the L5 DRG neuron of animals at different stages of life. The average level of signaling intensity was measured. The signaling intensity of the neurofilament is not significantly different in all groups.

A study conducted on sympathetic neurons showed that Akt acts as an important mediator for PI3K-dependent survival signals.[35] An injury in a nerve may activate the PI3K-Akt pathway [36]. This is evident by the robust increase of pAkt level in the DRGs and motoneurons following injury [19,22,33] probably via the PI3K-Akt pathway.[20] The activation of this pathway will initiate nerve regeneration and neurite elongation.[20] Without PI3K, activation of Akt will decrease, hence, attenuate the neurite outgrowth.[37]

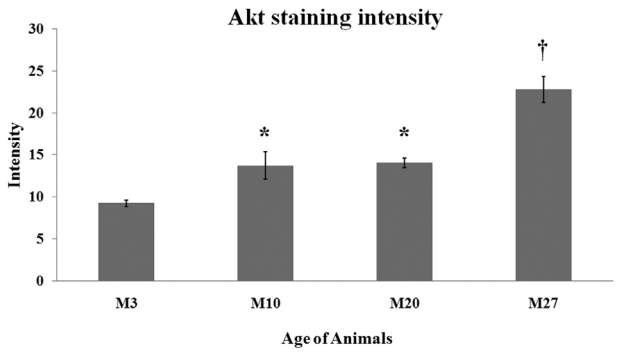

Fig. 3. The signaling of Akt in the L5 DRG neuron of animals at different stages of life. The average level of signaling intensity was measured. Low level of Akt signaling is detected in the M3 neurons. The intensity is higher in M10 and M20, showed the increase need of Akt for cells homeostasis. At advanced aged, M27, the signaling of Akt showed intense staining, significantly higher compared to the younger counterparts.
* Significantly different from M3 and M27.
† Significantly different from M3, M10 and M20.

There are several studies that showed decreasing levels of Akt activation and phosphorylation in aging animals, like in hepatocytes,[23,24] muscles[38,39] and artery.[27] In aged subjects, the circulating level of IGF-1 and in muscular tissue is reduced, which later leads to muscle atrophy.[40,41] The reduced level of IGF-1 could be one of the reasons of attenuated Akt expression in aging animals. On the other hand, previous studies showed that the activation of Akt and its phosphorylated form is increased in aged animals.[25,26,39] Meanwhile, our study showed that the signaling intensity of Akt is increased after M10. However, the spurt of pAkt signaling intensity is seen at M27. It is suggested that the activity of Akt in the DRG neurons is increased as the animals aged.

Furthermore, our study showed that the translocation of pAkt occurred in aged DRG in both large- and small-sized neurons. The translocation of pAkt into the nuclei of the DRG has been seen in the axotomized and inflamed nerve.[22] This translocation is induced by NGF.[42,43] In addition, PI3K is suggested to be directly involved in the translocation since the introduction of cells to LY294002, a PI3K inhibitor, prevents the translocation of Akt and pAkt into the nucleus.[18,42,43] Previously, it has been suggested that phosphorylation of Akt has been used as an indicator for PI3K activity.[44] In this study, in addition to Akt and phosphorylated Akt, it is indicated that IGF-1 and PI3K are also increased to directly initiate the activation of Akt and for translocation of pAkt into the

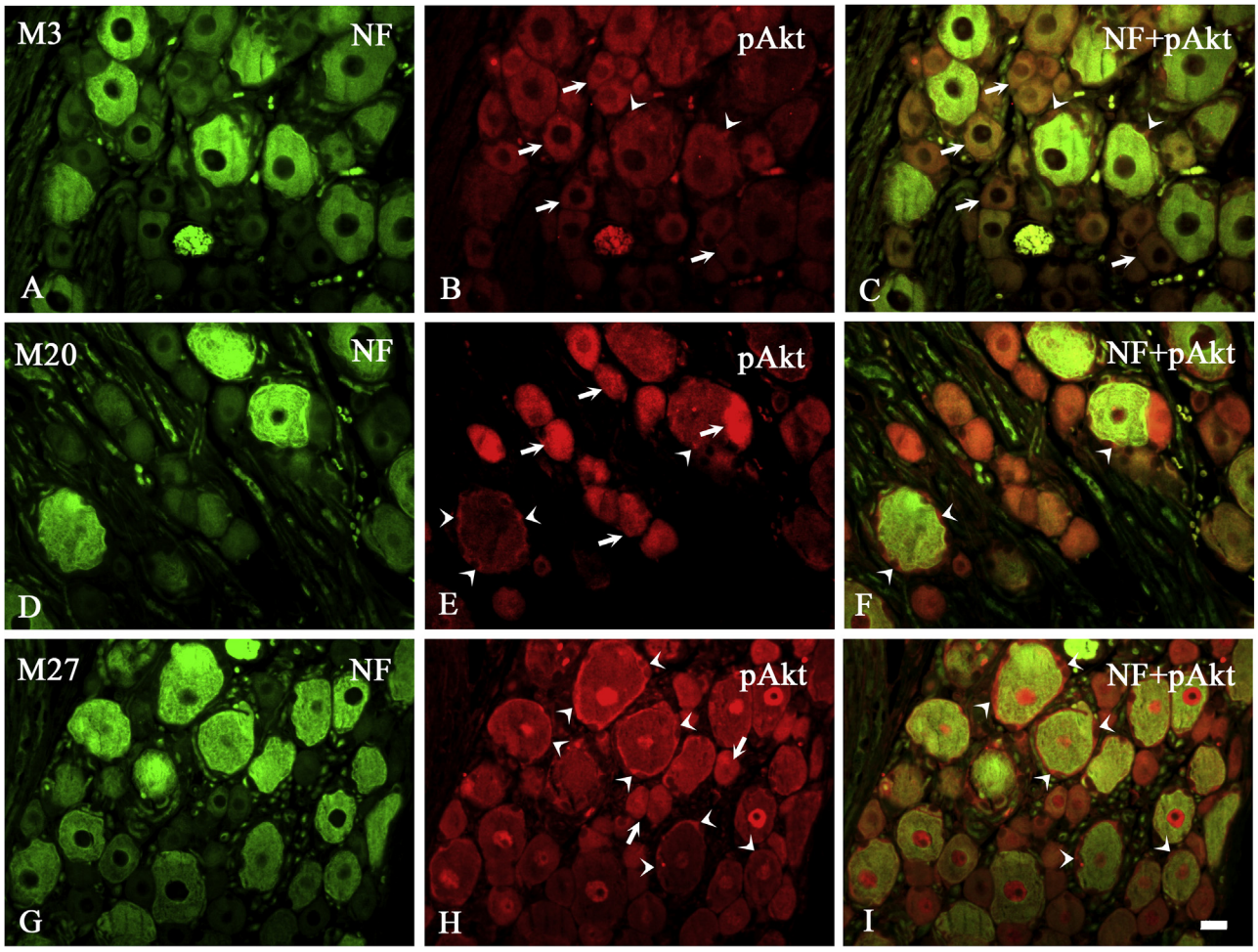

Fig. 4. Phosphorylated Akt (pAkt) immunoreactive was detected in almost all DRG neurons, with higher intensity in small-sized non-NF-immunoreactive neurons. At advanced aged, the pAkt is seen to be localized in the nucleus of all neurons (G–I). A–I: show the results of double immunofluorescence staining in L5 DRG between pAkt (CY3 red; B, E, H) and neurofilament (NF) (FITC Green; A, D, G) at the age of M3 (A–C), M20 (D–F) and M27 (G–I). C, F and I show images merged for NF and pAkt. The results showed that pAkt is localized in the cytoplasm of majority of cell neurons. However, in advanced aged animals, the pAkt has been translocated into the nuclei, indicating that higher degree of activity is occurring in the nuclei. The signaling of pAkt is also intense in the SGCs of the NF-immunoreactive neurons. Arrow – Small-sized neurons, arrowhead-satellite glial cells (SGCs), Scale bar A–I: 20 μm.

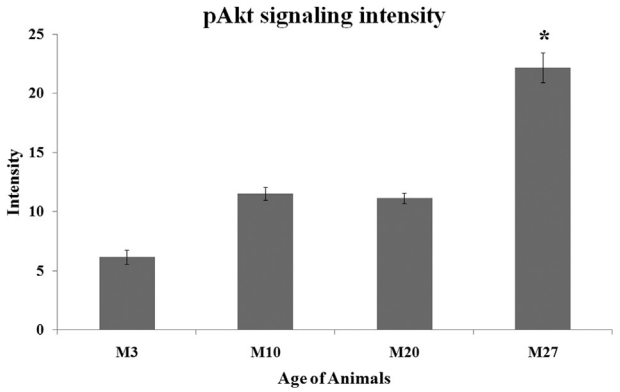

Fig. 5. The signaling of pAkt in the L5 DRG neuron of animals at different stages of life. The average level of signaling intensity was measured. Low level of pAkt signaling is detected in the M3, M10 and M20 neurons. At advanced aged, M27, the signaling of pAkt showed intense staining, significantly higher compared to the younger counterparts.
* Significantly different from M27; $p < 0.05$.

nuclei. However, more studies on the signaling of IGF-1, PI3K and their related kinases in the pathway are needed to confirm the speculation.

Satellite glial cells (SGCs) have been mostly ignored by researchers until a couple of decades ago. Since then, studies have been conducted on the role of the SGCs in relation to the neuronal proper functions. Pannese [45] described that all neuronal cell bodies are enveloped by a SGCs sheath. Under normal condition, the SGCs are able to meet the metabolic requirement of their respective neurons.[46] However, in extremely advanced aged, the ability of the SGCs to produce energy is decreased, hence the ability to support their associated neurons is also reduced.[47,48] The inability of SGCs to produce sufficient energy may be due to the structural changes of the mitochondria that occurred in advanced aged animals.[48,49] In this study, it is speculated that as the animals aged, the signaling of the pAkt is increased SGCs. Unfortunately, the association between the signaling of pAkt in the SGCs and neuronal Akt activity of aged animals is unknown. More study is needed to conclude the relation between the activation of Akt in the SGCs, neuronal cell bodies and aging. Our finding may perhaps contribute to the study of protein kinases influence on aging process particularly in peripheral nervous system and may add a value on the research for neurodegenaration therapy.

5. Conclusion

In conclusion, the expression of Akt and the signaling of pAkt are suggested to be the indicators that more activities are taking place in the DRG as a response to aging. The type of activities is unknown given that our study is limited to the signaling of Akt and pAkt. Furthermore, the activation of Akt in the SGCs of aged ganglia needs further investigation. In addition, there are other relevant participants suggested to be involved in the pathway, including IGF-1 and PI3K.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

This project was carried out with the financial support from Ministry of Higher Education Malaysia under the cluster of University Malaya research Grant (RP005B-13AFR) and Postgraduate Research Fund (PG103-2015A).

We would like to thank Koh Sui Lin, Robiah Bakar and the staff of Department of Anatomy, Faculty of Medicine, University of Malaya for technical supports.

References

1. Chakour MC, Gibson SJ, Bradbeer M, Helme RD. The effect of age on A delta- and C-fibre thermal pain perception. *Pain*. 1996;64(1):143–152.
2. Fundin BT, Bergman E, Ulfhake B. Alterations in mystacial pad innervation in the aged rat. *Exp Brain Res*. 1997;117(2):324–340.
3. Edwards RR, Fillingim RB. Age-associated differences in responses to noxious stimuli. *J Gerontol A Biol Sci Med Sci*. 2001;56(3):M180–M185.
4. Elder GA, Friedrich Jr. VLJr., Margita A, Lazzarini RA. Age-related atrophy of motor axons in mice deficient in the mid-sized neurofilament subunit. *J Cell Biol*. 1999;146(1):181–192.
5. Hashizume K, Kanda K. Differential effects of aging on motoneurons and peripheral nerves innervating the hindlimb and forelimb muscles of rats. *Neurosci Res*. 1995;22(2):189–196.
6. Falkenstein M, Yordanova J, Kolev V. Effects of aging on slowing of motor-response generation. *Int J Psychophysiol*. 2006;59(1):22–29.
7. Kallio J, Avela J, Moritani T, et al. Effects of ageing on motor unit activation patterns and reflex sensitivity in dynamic movements. *J Electromyogr Kinesiol*. 2010;20(4):590–598.
8. Lauretani F, Bandinelli S, Bartali B, et al. Axonal degeneration affects muscle density in older men and women. *Neurobiol Aging*. 2006;27(8):1145–1154.
9. Parhad IM, Scott JN, Cellars LA, Bains JS, Krekoski CA, Clark AW. Axonal atrophy in aging is associated with a decline in neurofilament gene expression. *J Neurosci Res*. 1995;41(3):355–366.
10. Bergman E, Johnson H, Zhang X, Hokfelt T, Ulfhake B. Neuropeptides and neurotrophin receptor mRNAs in primary sensory neurons of aged rats. *J Comp Neurol*. 1996;375(2):303–319.
11. Ulfhake B, Bergman E, Edstrom E, et al. Regulation of neurotrophin signaling in aging sensory and motoneurons: dissipation of target support? *Mol Neurobiol*. 2000;21(3):109–135.
12. Gonzalez E, McGraw TE. The Akt kinases: isoform specificity in metabolism and cancer. *ABBV Cell Cycle*. 2009;8(16):2502–2508.
13. Alessi DR, Andjelkovic M, Caudwell B, et al. Mechanism of activation of protein kinase B by insulin and IGF-1. *EMBO J*. 1996;15(23):6541–6551.
14. Jones PF, Jakubowicz T, Pitossi FJ, Maurer F, Hemmings BA. Molecular cloning and identification of a serine/threonine protein kinase of the second-messenger subfamily. *Proc Natl Acad Sci U S A*. 1991;88(10):4171–4175.
15. Cheng JQ, Godwin AK, Bellacosa A, et al. AKT2, a putative oncogene encoding a member of a subfamily of protein-serine/threonine kinases, is amplified in human ovarian carcinomas. *Proc Natl Acad Sci U S A*. 1992;89(19):9267–9271.
16. Brodbeck D, Cron P, Hemmings BA. A human protein kinase Bgamma with regulatory phosphorylation sites in the activation loop and in the C-terminal hydrophobic domain. *J Biol Chem*. 1999;274(14):9133–9136.
17. Read DE, Gorman AM. Involvement of Akt in neurite outgrowth. *Cell Mol Life Sci*. 2009;66(18):2975–2984.
18. Andjelkovic M, Alessi DR, Meier R, et al. Role of translocation in the activation and function of protein kinase B. *J Biol Chem*. 1997;272(50):31515–31524.
19. Naidu M, David RP, Asher R, Fawcett J. Expression of akt and MAPK in the normal and regenerating peripheral nerves and their dorsal root ganglia. *Malaysian J Biochem Mol Biol*. 2009;17(1):16–19.
20. Namikawa K, Honma M, Abe K, et al. Akt/protein kinase B prevents injury-induced motoneuron death and accelerates axonal regeneration. *J Neurosci*. 2000;20(8):2875–2886.
21. Sun RQ, Tu YJ, Yan JY, Willis WD. Activation of protein kinase B/Akt signaling pathway contributes to mechanical hypersensitivity induced by capsaicin. *Pain*. 2006;120(1–2):86–96.
22. Shi TJ, Huang P, Mulder J, Ceccatelli S, Hokfelt T. Expression of p-Akt in sensory neurons and spinal cord after peripheral nerve injury. *Neurosignals*. 2009;17(3):203–212.
23. Ikeyama S, Kokkonen G, Shack S, Wang XT, Holbrook NJ. Loss in oxidative stress tolerance with aging linked to reduced extracellular signal-regulated kinase and Akt kinase activities. *FASEB J*. 2002;16(1):114–116.
24. Shay KP, Hagen TM. Age-associated impairment of Akt phosphorylation in primary rat hepatocytes is remediated by alpha-lipoic acid through PI3 kinase, PTEN, and PP2A. *Biogerontology*. 2009;10(4):443–456.
25. Wu M, Katta A, Gadde MK, et al. Aging-associated dysfunction of Akt/protein kinase B: S-nitrosylation and acetaminophen intervention. *PLoS One*. 2009;4(7):e6430.
26. Gaugler M, Brown A, Merrell E, DiSanto-Rose M, Rathmacher JA. Reynolds THt PKB signaling and atrogene expression in skeletal muscle of aged mice. *J Appl Physiol (1985)*. 2011;111(1):192–199.
27. Trott DW, Luttrell MJ, Seawright JW, Woodman CR. Aging impairs PI3K/Akt signaling and NO-mediated dilation in soleus muscle feed arteries. *Eur J Appl Physiol*. 2013;113(8):2039–2046.
28. Schmalbruch H. The number of neurons in dorsal root ganglia L4-L6 of the rat. *Anat Rec*. 1987;219(3):315–322.
29. Kovacheva EL, Hikim AP, Shen R, Sinha I, Sinha-Hikim I. Testosterone supplementation reverses sarcopenia in aging through regulation of myostatin, c-Jun NH2-terminal kinase, Notch, and Akt signaling pathways. *Endocrinology*. 2010;151(2):628–638.

30. Kimball SR, O'Malley JP, Anthony JC, Crozier SJ, Jefferson LS. Assessment of biomarkers of protein anabolism in skeletal muscle during the life span of the rat: sarcopenia despite elevated protein synthesis. *Am J Physiol Endocrinol Metab*. 2004;287(4):E772–80.
31. Rotwein P, Wilson EM. Distinct actions of Akt1 and Akt2 in skeletal muscle differentiation. *J Cell Physiol*. 2009;219(2):503–511.
32. Astle MV, Hannan KM, Ng PY, et al. AKT induces senescence in human cells via mTORC1 and p53 in the absence of DNA damage: implications for targeting mTOR during malignancy. *Oncogene*. 2012;31(15):1949–1962.
33. Pezet S, Spyropoulos A, Williams RJ, McMahon SB. Activity-dependent phosphorylation of Akt/PKB in adult DRG neurons. *Eur J Neurosci*. 2005;21 (7):1785–1797.
34. Sun R, Yan J, Willis WD. Activation of protein kinase B/Akt in the periphery contributes to pain behavior induced by capsaicin in rats. *Neuroscience*. 2007;144(1):286–294.
35. Crowder RJ, Freeman RS. Phosphatidylinositol 3-kinase and Akt protein kinase are necessary and sufficient for the survival of nerve growth factor-dependent sympathetic neurons. *J Neurosci*. 1998;18(8):2933–2943.
36. Xu JT, Tu HY, Xin WJ, Liu XG, Zhang GH, Zhai CH. Activation of phosphatidylinositol 3-kinase and protein kinase B/Akt in dorsal root ganglia and spinal cord contributes to the neuropathic pain induced by spinal nerve ligation in rats. *Exp Neurol*. 2007;206(2):269–279.
37. Jones DM, Tucker BA, Rahimtula M, Mearow KM. The synergistic effects of NGF and IGF-1 on neurite growth in adult sensory neurons: convergence on the PI 3-kinase signaling pathway. *J Neurochem*. 2003;86(5):1116–1128.
38. Li M, Li C, Parkhouse WS. Age-related differences in the des IGF-I-mediated activation of Akt-1 and p70 S6 K in mouse skeletal muscle. *Mech Ageing Dev*. 2003;124(7):771–778.
39. Paturi S, Gutta AK, Katta A, et al. Effects of aging and gender on muscle mass and regulation of Akt-mTOR-p70s6k related signaling in the F344BN rat model. *Mech Ageing Dev*. 2010;131(3):202–209.
40. Severgnini S, Lowenthal DT, Millard WJ, Simmen FA, Pollock BH, Borst SE. Altered I.G.F-I and IGFBPs in senescent male and female rats. *J Gerontol A Biol Sci Med Sci*. 1999;54(3):B111–5.
41. Sandri M, Barberi L, Bijlsma AY, et al. Signalling pathways regulating muscle mass in ageing skeletal muscle: the role of the IGF1-Akt-mTOR-FoxO pathway. *Biogerontology*. 2013;14(3):303–323.
42. Borgatti P, Martelli AM, Tabellini G, Bellacosa A, Capitani S, Neri LM. Threonine 308 phosphorylated form of Akt translocates to the nucleus of PC12 cells under nerve growth factor stimulation and associates with the nuclear matrix protein nucleolin. *J Cell Physiol*. 2003;196(1):79–88.
43. Xuan Nguyen TL, Choi JW, Lee SB, et al. Akt phosphorylation is essential for nuclear translocation and retention in NGF-stimulated PC12 cells. *Biochem Biophys Res Commun*. 2006;349(2):789–798.
44. Watanabe H, Saito H, Rychahou PG, Uchida T, Evers BM. Aging is associated with decreased pancreatic acinar cell regeneration and phosphatidylinositol 3-kinase/Akt activation. *Gastroenterology*. 2005;128(5):1391–1404.
45. Pannese E. The structure of the perineuronal sheath of satellite glial cells (SGCs) in sensory ganglia. *Neuron Glia Biol*. 2010;6(1):3–10.
46. Ledda M, Barni L, Altieri L, Pannese E. The Golgi apparatus of satellite cells associated with spinal ganglion neurons: changes with age in the rabbit. *J Submicrosc Cytol Pathol*. 2003;35(3):267–270.
47. Martinelli C, Sartori P, Ledda M, Pannese E. Age-related quantitative changes in mitochondria of satellite cell sheaths enveloping spinal ganglion neurons in the rabbit. *Brain Res Bull*. 2003;61(2):147–151.
48. Martinelli C, Sartori P, Ledda M, Pannese E. Mitochondria in perineuronal satellite cell sheaths of rabbit spinal ganglia: quantitative changes during life. *Cells Tissues Organs*. 2007;186(2):141–146.
49. Martinelli C, Sartori P, De Palo S, Ledda M, Pannese E. The perineuronal glial tissue of spinal ganglia. Quantitative changes in the rabbit from youth to extremely advanced age. *Anat Embryol (Berl)*. 2006;211(5):455–463.