Depletion of Endothelial Krüppel-like Factor 4 (KLF4) Drives Age-Related Neurovascular Dysfunction and Neuropsychiatric Impairment

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Abstract

Background: As we get older, various aspects of our bodies start to decline, including brain function. The mechanisms behind the decline in brain function due to aging are still not well understood. The blood-brain barrier (BBB) is crucial for maintaining brain health and homeostasis across the lifespan by controlling both passive and active exchange of materials between the blood and brain, as well as neurovascular coupling, which is instantly calibrates delivery of blood to brain regions to precisely match their energy demand. Both aging and neurodegenerative disorders are associated with deterioration of endothelial cells, the primary component of the BBB. Endothelial cells are highly enriched in the Kruppel-like factor 4 (KLF4) transcription factor, which is also reduced in expression with aging.

Study Design and Methodology: Through neurobehavioral tests, two-photon microscopy, histological analysis, and single-cell RNA sequencing, we compared the brain health of endothelial-specific KLF4 knockout (EC-K4KO) mice compared to wild-type littermates across the lifespan.

Results: We observed that EC-K4KO mice display accelerated brain aging, including accelerated BBB deterioration, neurodegeneration, neuroinflammation, and cognitive decline. Single-cell RNA sequencing from brain endothelial cells of EC-K4KO mice revealed aberrantly high expression of adaptive and innate immune response genes. In addition, live animal brain imaging across the lifespan revealed accelerated BBB leakage, decreased blood flow, and impaired neurovascular coupling.

Conclusion: We show that the specific loss of KLF4 from endothelial cells promotes breakdown of the blood-brain barrier (BBB), a key aspect of brain aging, which is associated with vascular and neuronal pathology leading to cognitive decline. EC-K4KO mice provide a model of microvascular dysfunction in aging that was previously lacking in the field of brain health. Endothelial KLF4 or its downstream pathways could offer a novel strategy to protect the health and function of the aging brain.

Endothelial KLF4 Depletion Accelerates Aging-Related Basal and Stimulus-Driven Neurovascular Function

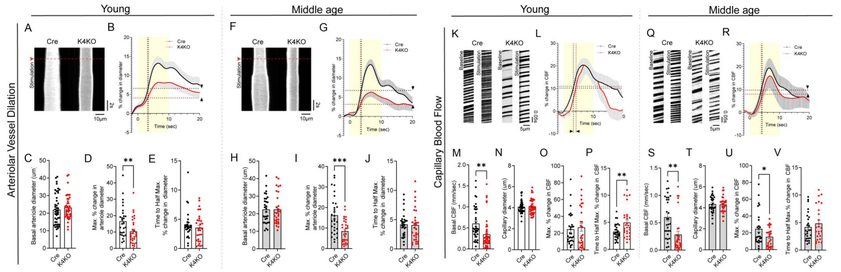

Figure 2: (A, B) Representative two-photon line-scan images (A) and average time course of arteriolar dilation (B) before and after hind-limb stimulation in Young Cre and Young K4KO. (C) Basal arteriolar diameter was not different between groups. (D) Maximum % change in arteriolar diameter in response to stimulation was significantly reduced in Young K4KO mice. (E) Time to half maximum % change in diameter was not different between groups. (F-J) Representative two-photon line-scan images (F) and average time course of arteriolar dilation (G) before and after hind-limb stimulation in Middle-age Cre and Middle-age K4KO. Maximum % change in arteriolar diameter in response to stimulation remained significantly reduced in Middle-age K4KO mice (I) without any difference in basal arteriolar diameter (H) and time to half maximum % change in diameter (J) when compared to Middle-age Cre. (K-L) Representative two-photon line-scan images (K) and average time course of capillary blood flow (L) during baseline and after hind-limb stimulation in Young Cre and Young K4KO. (M, N) Basal CBF (M) is significantly reduced in Young K4KO mice without any difference in Capillary diameter (N) between groups. (O, P) Maximum % change in CBF (O) was the same between groups however time to half maximum % change in CBF (P) was slower in Young K4KO mice. (Q-V) Representative two-photon line-scan images (Q) and average time course of capillary blood flow (R) during baseline and after hind-limb stimulation in Middle-age Cre and Middle-age K4KO. Remaining panel descriptors are same as K-P. Basal CBF (S) remained slower in Middle-age K4KO without any difference in capillary diameter (T) and time to half maximum % change in CBF (V) however in Middle-age K4KO the maximum % change in CBF (U) was significantly reduced compared to Middle-age Cre. All data shown are Average (±SEM). Individual data points represent individual blood vessels with black filled circles from Cre mice, red closed circles from K4KO mice. Significance was tested using Two-tailed T-test. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Endothelial KLF4 Depletion Accelerates Aging-Related Vascular and Neuronal Degeneration

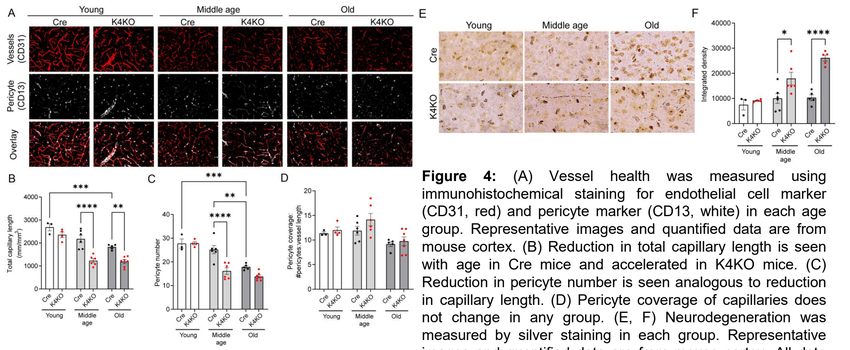

Figure 4: (A) Vessel health was measured using immunohistochemical staining for endothelial cell marker (CD31, red) and pericyte marker (CD13, white) in each age group. Representative images and quantified data are from mouse cortex. (B) Reduction in total capillary length is seen with age in Cre mice and accelerated in K4KO mice. (C) Reduction in pericyte number is seen analogous to reduction in capillary length. (D) Pericyte coverage of capillaries does not change in any group. (E, F) Neurodegeneration was measured by silver staining in each group. Representative images and quantified data are from mouse cortex. All data shown are Average (±SEM). Individual data points represent individual animals with black filled circles as Cre and red closed circles as K4KO. Significance was tested using One-Way ANOVA and Bonferroni post hoc analysis. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Endothelial KLF4 Deletion Accelerates Age-Related Behavioral Decline

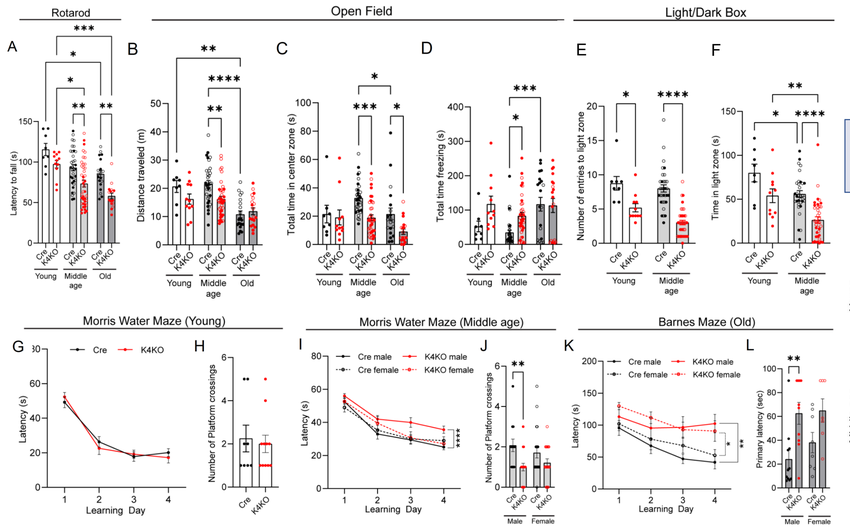

Figure 1: (A) Results from Rotarod test imply increased aging in K4KO mice (B) Distance travelled in an Open field apparatus reduces with age in control mice while only middle-aged K4KO mice show a deficit (C, D) Anxiety-like behavior tested in an Open field shows K4KO are more anxious (E, F) Light/Dark box test was used to measure anxiety-like behavior and showed that K4KO mice have increased anxiety (G, H) Learning and memory was tested in Young K4KO mice in a Morris Water Maze, with no significant difference compared to age-matched counterparts (I, J) Learning and memory was tested in Middle-aged K4KO mice in a Morris Water Maze (K, L) Learning and memory was tested in Old K4KO mice in a Barnes Maze test. All data shown are Average (±SEM). Individual data points represent individual animals with black filled circles as Cre males, black open circles as Cre females, red closed circles as K4KO males and red open circles as K4KO females (except panel G, I, K which are average values). Significance was tested using One-Way ANOVA and Bonferroni post hoc analysis for all panels except G, I and K which were analyzed using Two-way ANOVA and Bonferroni post hoc analysis. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Endothelial KLF4 Depletion Accelerates Aging-Related Blood-Brain Barrier Deterioration After Middle-Age.

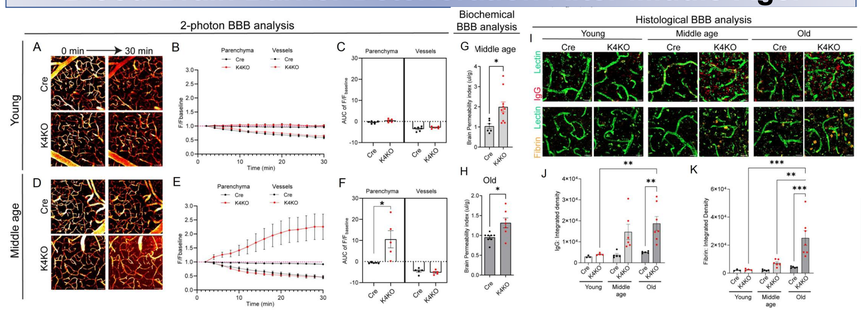

Figure 3: (A, D) Representative two-photon z-projection images from the same field of view 2 and 30 minutes after retro-orbital injection of 3kD fluorescent-dextran in Young (A) and Middle-age (D) Cre and K4KO mice. (B, E) Average time course of change in parenchymal and vessel fluorescent intensity. (C, F) Area under the Curve (AUC) was calculated from the time course data showing a significant accumulation of 3kD fluorescent-dextran in parenchyma of Middle-age K4KO mice (F). (G, H) BBB permeability to 3kD fluorescent-dextran was measured using a biochemical assay in middle-age (G) and old-age (H). (I) BBB permeability to endogenous plasma proteins was measured using immunohistochemical assay in each age group. Representative images and quantified data are from mouse cortex. (J, K) Cortical parenchymal IgG (J) and Fibrin (K) levels are significantly increased by age in K4KO mice and significantly increased in Old K4KO compared to Old Cre. All data shown are Average (±SEM). Individual data points represent individual animals with black filled circles as Cre and red closed circles as K4KO. Significance was tested using Two-tailed T-test for panels C, F, G, H and One-Way ANOVA and Bonferroni post hoc analysis for panels J and K. *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

Endothelial KLF4 Depletion Causes an Aging Transcription Profile in the Brain of Young Mice

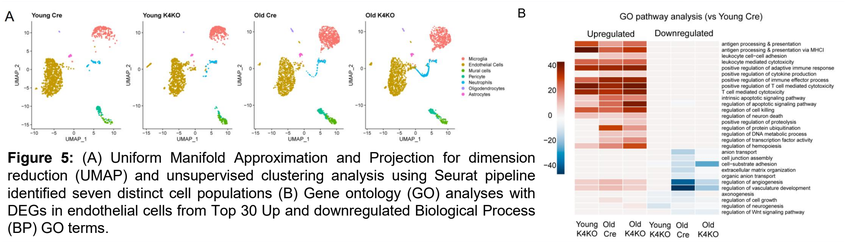

Figure 5: (A) Uniform Manifold Approximation and Projection for dimension reduction (UMAP) and unsupervised clustering analysis using Seurat pipeline identified seven distinct cell populations (B) Gene ontology (GO) analyses with DEGs in endothelial cells from Top 30 Up and downregulated Biological Process (BP) GO terms.

Conclusions

Our data suggest that loss of KLF4 expression in endothelial cells drives aging-related BBB deterioration and neurodegeneration. Endothelial cell KLF4 signaling with age may be a novel therapeutic target to preserve the health and function of the aging brain.

Acknowledgments

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