

Supplementary information

Title:

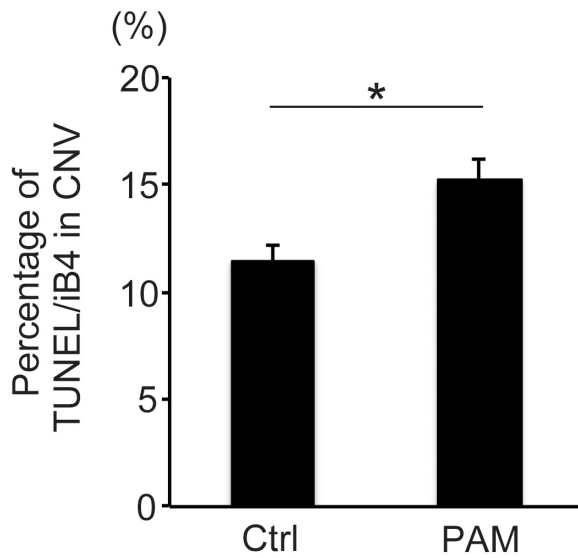
Plasma-activated medium suppresses choroidal neovascularization in mice: a new therapeutic concept for age-related macular degeneration

Authors:

Fuxiang Ye, Hiroki Kaneko, Yosuke Nagasaka, Ryo Ijima, Kae Nakamura,

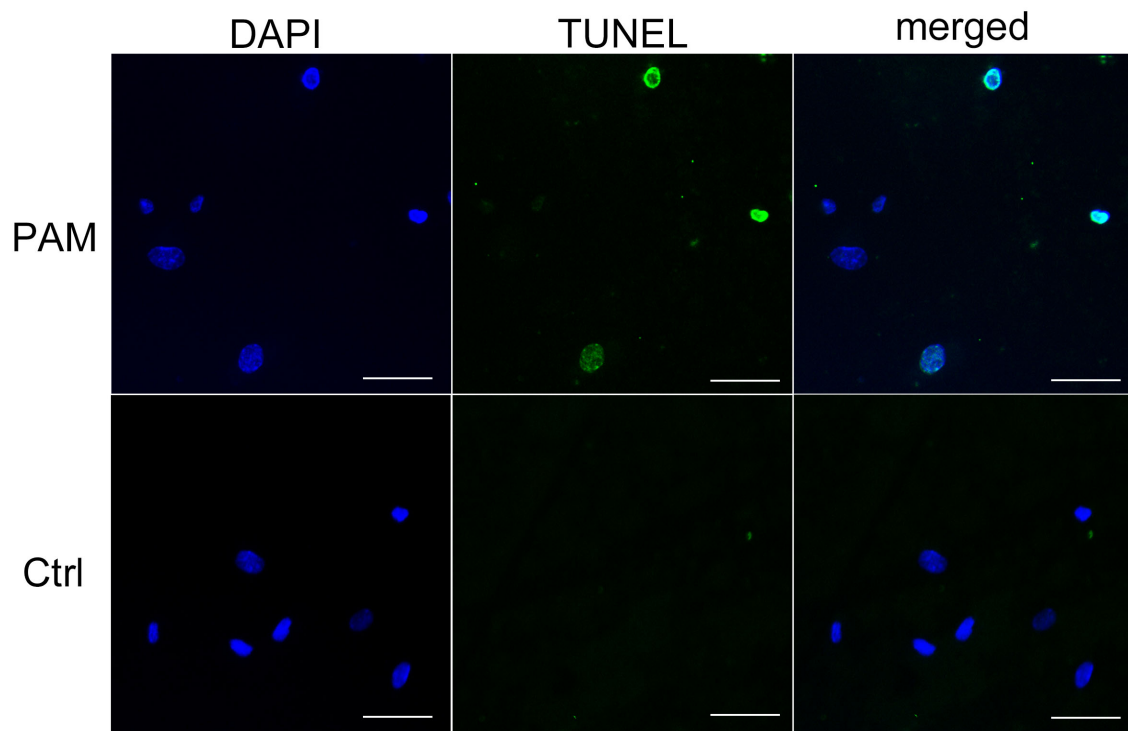
Masatoshi Nagaya, Kei Takayama, Hiroaki Kajiyama, Takeshi Senga,

Hiromasa Tanaka, Masaaki Mizuno, Fumitaka Kikkawa, Masaru Hori, Hiroko Terasaki



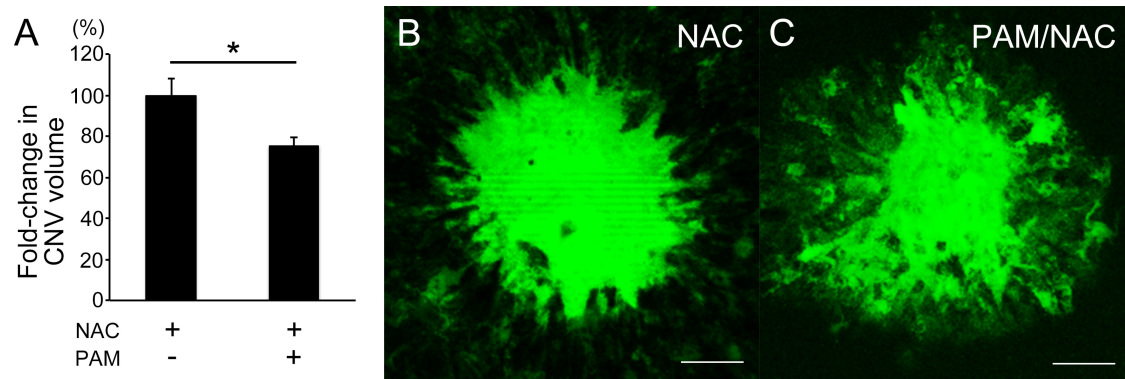
Supplementary Figure 1 – TUNEL-positive area within CNV area in eyes injected with PAM.

The percentage of the TUNEL-positive area within the isolectin B4 (iB4)-positive CNV area was calculated in eyes injected with PAM, in comparison with those injected with control PBS. The TUNEL/iB4 percentage was $11.5 \pm 0.7\%$ (n=8) in control eyes, whereas it was $15.2 \pm 1.0\%$ (n=8) in PAM-injected eyes. The TUNEL/iB4 percentage was increased by 32% ($P = 0.012$). * $P < 0.05$.



Supplementary Figure 2 – PAM induced HREC apoptosis

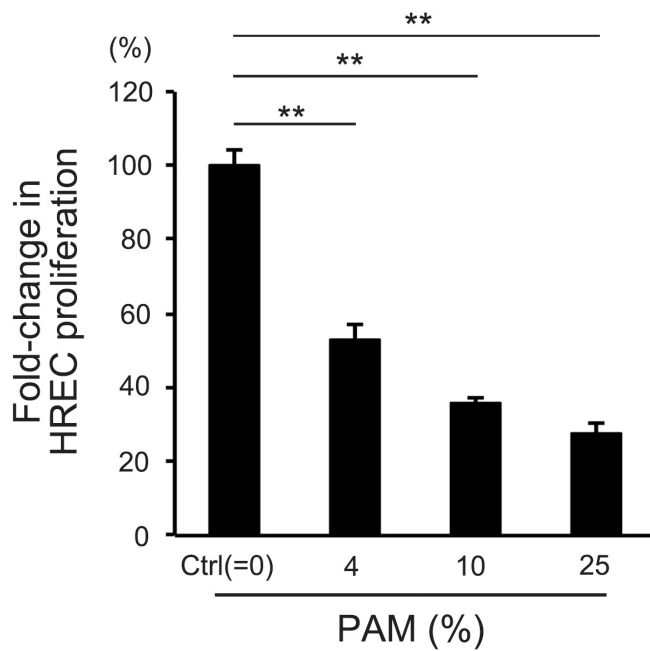
After 4 h of incubation with PAM, HRECs were fixed in 4% PFA and stained with FITC-TUNEL and DAPI. HRECs treated with PAM showed TUNEL-positivity, whereas HRECs exposed only to control medium did not. Scale bar = 50 μ m



Supplementary Figure 3 – Laser-CNV comparison in PAM/NAC-injected vs.

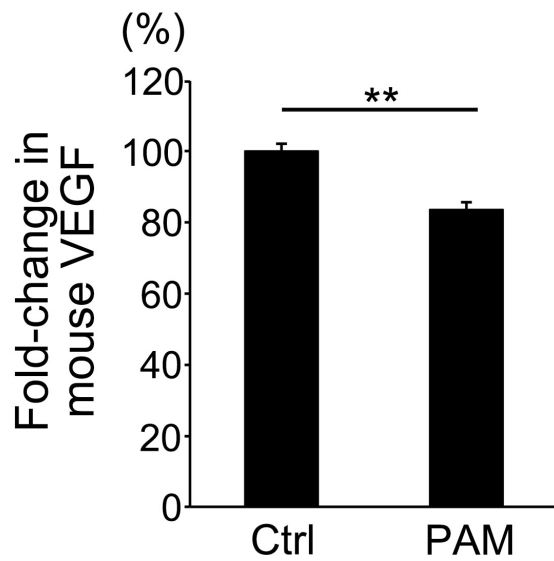
NAC-injected eyes

Laser-CNV volume in the eyes injected with PAM/NAC was compared with those injected with PAM only. The CNV volume in eyes injected with PAM and NAC was less than that in eyes injected with NAC only ($1.00\% \pm 0.08\%$ vs. $0.75\% \pm 0.04\%$, $P = 0.027$, $n = 18$ and 17 , respectively). **(B, C)** Representative images of laser-CNV in NAC-injected (B) and PAM/NAC-injected eyes (C). Scale bar = $50 \mu\text{m}$, $*P < 0.05$.



Supplementary Figure 4 – Suppressed cell proliferation by PAM

After 4 h of incubation with PAM, the proliferation ability of human retinal endothelial cells (HRECs) was clearly inhibited compared to control ($P = 6.28 \times 10^{-7}$, Kruskal–Wallis test). The different concentrations of PAM (4 %, 10 %, and 25 % concentrations) dose dependently inhibited cell proliferation by 47% (0.53 ± 0.04 , $n = 10$, $P = 4.4 \times 10^{-4}$) in 4 % PAM, by 64 % (0.36 ± 0.01 , $n = 10$, $P = 4.6 \times 10^{-4}$) in 10 % PAM, and by 72 % (0.28 ± 0.03 , $n = 10$, $P = 4.5 \times 10^{-4}$) in 25 % PAM compared to the control (=0% PAM) medium (1.00 ± 0.05 , $n = 10$, Steel’s test). $**P < 0.01$.



Supplementary Figure 5 – Reduced mouse VEGF expression in the RPE/choroid complex with CNV after PAM administration

Three days after the induction of laser-CNV and PAM injection, mouse vascular endothelial growth factor (VEGF) expression in the retinal pigment epithelium (RPE)/choroid complex was measured. Mouse VEGF in the RPE/choroid was reduced by 16% by PAM injection. (1.00 ± 0.22 vs. 0.84 ± 0.24 , $P = 0.0027$, $n = 7$). $**P < 0.01$.

Supplementary Video – Generation of PAM

Plasma-activated medium (PAM) was generated by exposure to a plasma source placed 2 mm over the PBS or culture medium in all studies.