Purinergic Signaling Modulates Survival/Proliferation of Human Dental Pulp Stem Cells

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Appendix

Materials and Methods

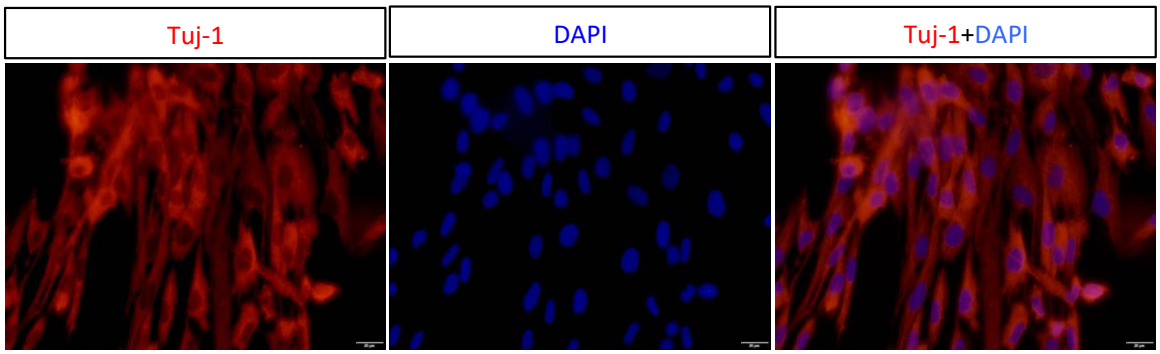
Immunocytochemical fluorescence staining

Fixed hDPSCs were first incubated with blocking solution (5% normal goat serum / 0.3% Triton X-100 in 1× PBS (phosphate-buffered saline)) for 2h at 4^{o}C and then incubated overnight with mouse, rat or rabbit antibodies against Tuj-1, Stro-1, CD44, P2X3, P2X7, P2Y2 (1:1000; Sigma-Aldrich), rabbit anti-Ki67 (1:400; NovusBio), mouse or rabbit anti-NTPDase2 or anti-NTPDase3 (1:500; generously provided by Dr. J. Sevigny at Laval University). After washing with 1× PBS, the slides were incubated for 2h with secondary antibodies conjugated with either Alexa Fluor 488 or Alexa Fluor 543 (1:200; Molecular Probes). Slides were then rinsed with 1× PBS and cover-slipped with a DAPI (4′, 6-diamidino-2-phenylindole) mounting medium. Control experiments were performed using the same protocol in the absence of primary antisera. Slides were viewed and captured using a confocal microscope (Olympus, Japan) or epi-fluorescence microscope.

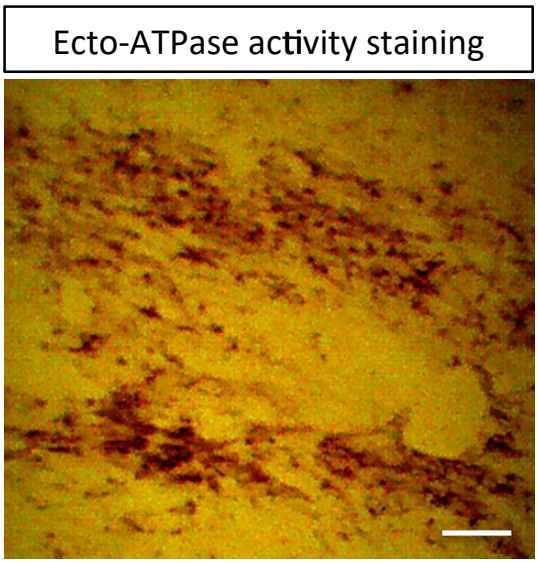
Enzymatic cytochemistry staining

To demonstrate functional ATPase activity, hDPSCs slides were fixed with 4% paraformaldehyde in PBS for 1 min. After washing with 1× PBS, hDPSCs were pre-incubated for 30 min at room temperature with Tris-maleate-sucrose buffer (0.25M sucrose, 50mM Tris-maleate, pH 7.4) containing 2mM $CaCl_2$. The enzyme reaction proceeded for 60 min at room temperature in a Tris-maleate-sucrose-buffered substrate solution (1mM ATP, 2mM $Pb(NO_3)$ 2.5mM $MnCl_2$, 2mM $CaCl_2$, 50mM Tris-maleate, pH 7.4, plus 0.25M sucrose) stabilized with 3% dextran T250 (Roth, Karlsruhe, Germany).

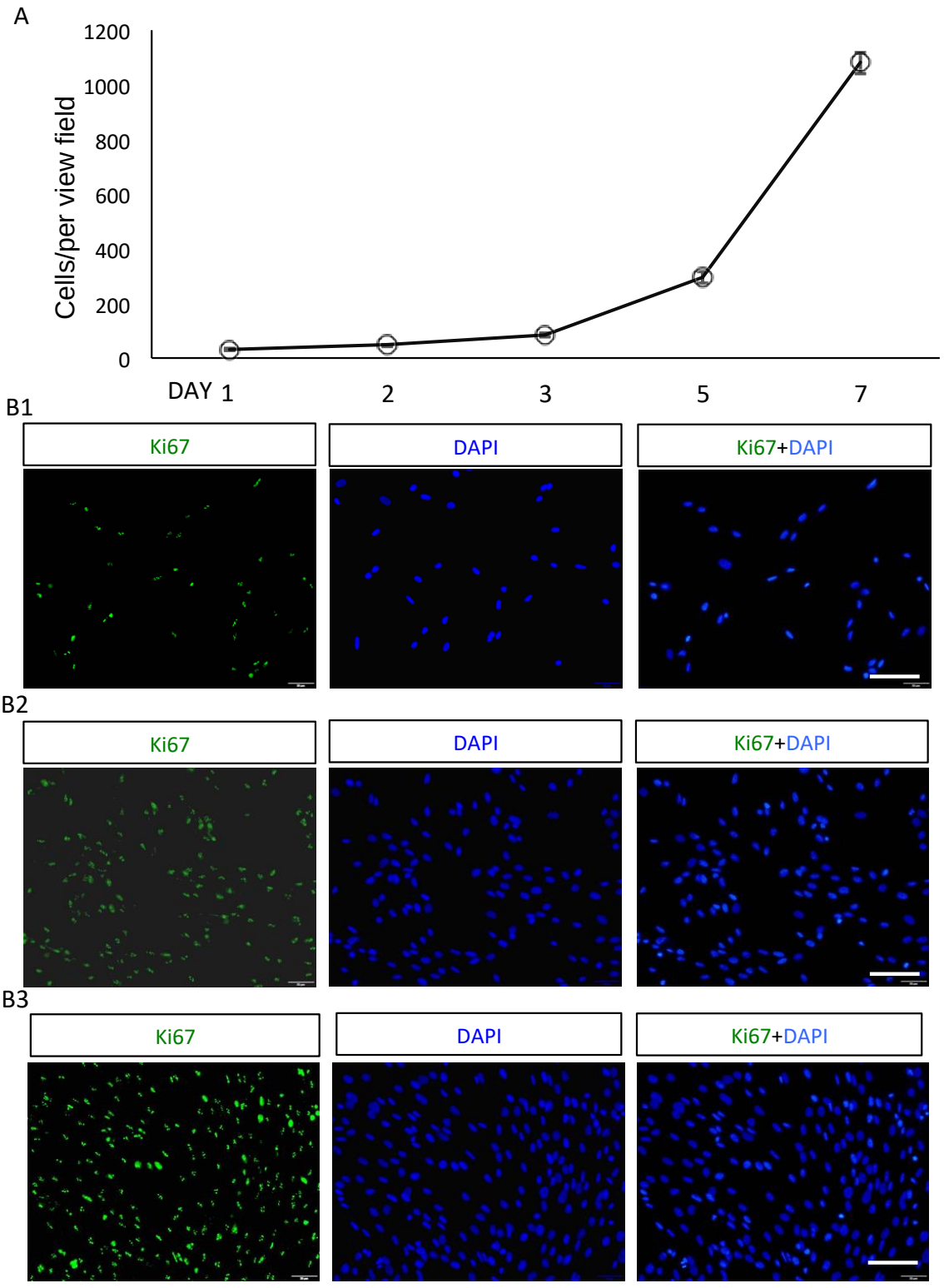
After washing with demineralized water, the lead orthophosphate, which is precipitated from ATP as a result of nucleotidase activity, was visualized as a brown deposit by incubating sections in an aqueous solution of $(NH_4)_2S$ (1% v/v) for 30 s. Subsequently, the slides were dehydrated in graded ethanol and mounted with Permount mounting medium (Fisher Scientific). To control for nonspecific lead precipitation, substrate was omitted from the incubation solution. Under these experimental conditions (pH 7.4), this assay detects ecto-nucleotidase (ecto-NTPDase) activity (Liu et al., 2012b)



Appendix Fig. 1 Expression of neuronal progenitor marker Tuj-1 in hDPSCs. Scale bar: 10μm.



Appendix Fig. 2 Enzymatic cytochemistry staining for ecto-ATPase activity was performed in cultured hDPSCs. Ecto-ATPase activity was detected in hDPSCs. Scale bar: 50μm.



Appendix Fig. 3 A: The growth curve of hDPSCs in culture from day 1 to day 7. B: Expression of Ki67 in cultured hDPSCs at day 3 (B1), day 5(B2) and day 7 (B3). Scale bar: 50μm.