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Y27632 promotes self-renewal in RA-treated mouse embryonic stem cell cultures by facilitating survival and proliferation of loosely attached cells

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Rho associated kinase (ROCK) inhibitor Y27632 can facilitate the survival of dissociated human embryonic stem (hES) cells and is also reported to be involved in renewal and neural differentiation of P19 cells as well as mouse embryonic stem (mES) cells. However, the mechanism of the dual-directional regulation effect of Y27632 on embryonic stem cell differentiation was not fully understood. In the present study, we found that Y27632 significantly potentiated prolonged culture of mouse embryonic stem cells with a delayed down regulation of Oct3/4. Concomitantly, Y27632 also induced the up-regulation of Oct3/4 protein level in all-trans retinoic acid (RA) treated cultures. Immunofluorescent staining showed that SSEA-1, a surface marker for pluripotent mES cells, was only expressed in a specific type of cells which were loosely attached to the plate. However, these loosely attached cells were seldom observed in RA treated cultures in the absence of Y27632. Based on the fact that the cell counts in RA treated cultures also increased in the presence of Y27632, we speculated that Y27632 facilitated the survival and proliferation of those cells. To confirm the hypothesis, we plated dissociated mES cells on low attachment culture dishes. As expected, the number of attached cells was greatly increased in RA combined with Y27632 treated groups, but in RA treated group did not. Together, our results indicate an indirect role of Y27632 in regulating renewal factors of embryonic stem cells. It will be great value to further reveal mechanisms of Y27632 on embryonic stem cells

Biography

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