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Thyrogenic, adipogenic, and osteogenic differentiation of adult rat, thyroid stem cells enriched by long-term adherent subculture

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We recently identified adult progenitor cells expressing multipotency markers in the rat thyroid (1). We have now studied these markers in primary cultures, thyrospheres, and adherent cells exhibiting features of side population / multilineage differentiation. Primary rat thyroid monolayers were immunolabeled / immunoblotted for ABCG2, Oct-3/4, HNF4a and Sca1. Thyrospheres were cytospinned and immunolabeled for Oct-3/4. Long-term subcultures were obtained by re-seeding monolayers at very low density, and growing them up to 5 months, using a starvation protocol to obtain colony forming unit (CFU)-like cultures. The latter were incubated with Hoechst (Hch) 33342 $\pm$ the ABCG2 inhibitor, verapamil (VE), to identify a side population, and immunostained for ABCG2, vimentin (VIM), and cytokeratin (CYT). Thyroid monolayers and CFU-like cultures were differentiated using TSH, adipogenic, and osteogenic media. Up to 1/4 cells from primary monolayers and thyrospheres resulted either ABCG2-, Oct-3/4-, HNF4a-, or Sca-1-positive. In contrast, in CFU-like cultures ABCG2 was detected in up to 1/3 cells, whereas VIM was ubiquitous, and CYT disappeared. Consistently, a side population was revealed by the Hch-VE staining. Finally, CFU-like cultures differentiated to cells containing either thyroglobulin, or red oil-, or alizarin red-positive deposits. We conclude that multilineage differentiation of our CFU-like thyroid cultures reveals enrichment of a thyroid stem cell population.

References

[1] Barbaro F. et al (2013). Adult stem/progenitor cells of the rat thyroid: side population distribution, intermediate filament expression, and long-term in vitro expansion I.J.A.E 118, (suppl 2) 19.

Keywords

Thyroid, stem cells, differentiation, multipotential.