

## Multipotent adult rat, thyroid stem cells can be differentiated to follicular thyrocyte, and hepatocyte-like cells in 2D and 3D culture systems

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We have recently characterized and differentiated towards endodermal and mesodermal lineages progenitor cells of the adult rat thyroid, expressing multipotency markers [1]. We have now assessed their clonogenicity, extent of side population, consistency of stem cell marker expression, and commitment to either follicular or hepatocyte-like lineages when in monolayer (2D), and suspension or Matrigel (3D). Colony forming unit (CFU)-like cultures were obtained by long-term subcultures of primary rat thyroid cells, under starvation conditions. CFU-like cultures seeded in Petri dishes by limiting dilution (1 cell / cm<sup>2</sup>) were observed to give rise to toluidine blue-positive, individual clones. In these cultures, quantitative densitometric analysis of immunoblotted Oct-3/4, Sca1, and GATA4 revealed an increase in stem cell markers ranging from 95% to 270% with respect to standard, primary thyroid cultures. In addition, using three different analytical techniques including DyeCycle Violet staining by flow cytometry, ABCG2 immunocytochemistry, and Hoechst 33342 histochemistry  $\pm$  the ABCG2 inhibitor, verapamil a side population involving 1-2% of CFU-like cultures was detected. Then, CFU-like cultures were differentiated using TSH, either in 2D or in 3D. Differentiated adherent cells resulted immunopositive for thyrocyte markers including thyroglobulin (TG), sodium-iodide symporter (NIS), and thyroperoxidase (TPO). Differentiation in suspension and in Matrigel gave rise to follicles with cells having ultrastructural features consistent with thyrocytes, and immunoreactivity (IR) for TG, NIS, and TPO. Finally, CFU-like cultures were differentiated in adherence to hepatocyte-like cells, resulting in pre-hepatocyte morphology, high periodic acid-Schiff reaction, and IR for  $\alpha$ -fetoprotein and albumin. We conclude that our CFU-like thyroid cultures are enriched with a multipotent, stem cell population whose hepatic differentiation capacity has been revealed for the first time.

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### References

- [1] Bassi E et al. (2014). Thyrogenic, adipogenic, and osteogenic differentiation of adult rat, thyroid stem cells enriched by long-term adherent subculture. *IJ.A.E* 119, (suppl to 1), 15, 2014.

### Keywords

Thyroid; stem cells; differentiation; multipotential.