

## Adult stem / progenitor cells of the rat thyroid: side population distribution, intermediate filament expression, and long-term *in vitro* expansion

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We recently identified adult stem / progenitor cells in the male rat thyroid, based on expression of the multipotency maker, ATP-binding cassette subfamily G member 2 (ABCG2) (1). To characterize these cells, we have now determined their distribution as a side population of the adult thyroid gland, identified their epithelial vs mesenchymal commitment by the presence of cytoplasmic intermediate filaments, and enriched their number using long-term, *in vitro* expansion of adherent elements. Sprague-Dawley male rats (50-75 gr) were used as thyroid donors. Following penthobarbital anesthesia rats were thyroidectomised, and primary cells prepared using enzymatic breaking of the gland. After 72 hs in standard monolayer culture, adherent cells were trypsinized, and either incubated for 90 min with the vital dye, Hoechst 33342 (Hch) ± the ABCG2 inhibitor, verapamil (VE, 150 mM) followed by cytospin (1300 RPM x 8 min) for single, double, and triple light microscopic immunocytochemistry (IC), or re-seeded ( $20 \times 10^3$  /  $\text{cm}^2$ ) in monolayer and grown up to 4 months, using a starvation protocol based on a single weekly change of culture medium (low glucose DMEM / 15 % FBS-FHS serum). Co-localization of nuclear Hch with immunoreactive (IR) ABCG2 (rabbit anti-human polyclonal antiserum, 1:300), IR-cytokeratin (CTK, mAb 1:200), and IR-vimentin (VIM, mAb 1:100) was assessed by the ABC and indirect fluorescence techniques, using DAB, FITC and TRITC as chromogens. Rat kidney, human keratinocyte cell line, NTCT 2544 (courtesy of C. Pellegrini), and primary mouse and human fibroblasts (courtesy of D. Mattioli) were used as positive controls for IC. A consistent increase in Hch-positive nuclei was observed in VE-treated cultures, as opposed to VE-untreated monolayers. In addition, an inverse staining relationship occurred between nuclear Hch and IR-CTK, as opposed to a direct relationship between nuclear Hch and IR-VIM. Co-localization of IR-ABCG2 with IR-CTK was seen in some cells, whereas that of IR-ABCG2 with IR-VIM was only occasionally detected. Finally, long-term expansion of primary thyrocytes resulted in 30% increase in IR-ABCG2 cells, as opposed to less than 1% IR-ABCG2 elements in standard culture. We conclude that ABCG2-positive cells of the rat thyroid are a side population of stem / progenitor elements, they are primarily committed to the epithelial phenotype, and can be enriched *in vitro* as adherent cells, suggesting clonal expansion.

### References

[1] Strusi V et al. (2012) IJAE 112 (2 supp): 184.

### Key words

Stem cells, thyroid, ABCG2, intermediate filaments.