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Identification of putative adult stem cells in the rat thyroid and their use in ex situ bioengineering

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Adult stem cells have been recently isolated from the human and mouse thyroid. Identification has been possible by their capacity to form floating cell spheroids or thyrospheres when primary cells are cultured in the absence of serum but presence of epidermal growth factor and basic fibroblast growth factor, as well as by the presence of stem cell markers like the breast cancer-resistant protein 1 (Bcrp1)/ATP-binding cassette subfamily G member 2 (ABCG2). Using this strategy, and an innovative *in vitro* growing system, we have attempted identification of stem / progenitor elements from the adult rat thyroid. Sprague-Dawley male rats (50-75 gr) were used as thyroid donors. After penthobarbital anesthesia rats were thyroidectomised, thyroids surgically excised, and primary cells prepared using enzymatic breaking. After 72 hs in standard monolayer culture, cells were trypsinized and either seeded (20 $x10^3/cm^2$) and grown for 8 days in a 3D Matrigel (12.5-50%) system using low-glucose DMEM and serum, or immediately cytospinned (1200 RPM x 5 min) for immunocytochemistry, or harvested and frozen with lysis buffer for Western blotting (WB). Bcrp1/ABCG2-immunoreactivity (IR) was detected using a rabbit anti-human, polyclonal antibody (1:500, Cell Signalling), and visualized either with the ABC technique and DAB as a chromogen, or with a chemiluminescence-based staining. The human plasmocytoma cell line, RPMI 8226 (B lymphocytes) and the acute lymphoblastic leukemia cell line CCRF-CEM (T lymphocytes) were used as positive and negative controls, respectively. Thyrosphere-like aggregates were transiently observed after initial monolayer expansion and, more consistently, at day 3 in 50% Matrigel culture, followed by rapid cell differentiation (days 4-8), including epithelial-mesenchymal transitions, formation of follicles and pavment layering. Similar differentiation changes were also detected after seeding of primary thyroid cells onto decellularized rat thyroid matrixes, as previously reported [1]. Less than 0.4% of cytospinned thyroid cells exhibited cytoplasmic Bcrp1/ABCG2-IR, and a band of around 72kD was detected by WB in cell lysates. We conclude that the thyroid of the adult rat contains a small population of stem / progenitor-like elements, likely contributing to the regenerative processes that occur during *ex situ* recellularization of acellular thyroid matrixes [1, 2].

References

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