5. Summary

In recent years, adult stem cells have become the subject of intense study. They have been analyzed in the context of normal physiology and have been associated with the development and propagation of some benign and malignant tumors. The presence of adult stem cells in human thyroid nodule tissues was first reported in our previous study. However, the isolation of thyroid stem cells has so far been hampered by the absence of a surface antigen that allows the separation by FACS. Therefore, the present study aimed to (1) achieve progresses in thyroid stem cell enrichment and purification, (2) analyze under which conditions quiescent stem cells derived from human nodules can be propagated to outgrow and (3) clarify if these cells have retained the capacity to differentiate into thyroid cells.

Taking advantage of the fact that stem cells express ABCG2 membrane transporters, thyroid stem cells from human nodules were separated by FACS analysis as a side population (SP), from a non-side population (non-SP) of cells that consisted of endodermal progenitor and differentiated cells, characterized by their typical gene profiles. Moreover, thyroid SP exhibited a characteristic morphology of adult stem cells, as compared with non-SP cells, after cytospin and Giemsa staining.

However, proliferation and differentiation of SP cells were limited by their poor viability and their quiescent state. To overcome this problem new isolation and stimulation techniques were employed. A small number of thyroid stem cells grew out from primary cell cultures in response to intense growth stimulation to form non-adherent, three-dimensional spheroid clones, which we termed ’thyro-spheres’. These spheres consisted of highly proliferating stem and progenitor cells with their characteristic expression profiles. Upon differentiation induction with serum and TSH, these sphere-forming cells grew as monolayer and differentiated into thyrocytes that expressed PAX8, thyroglobulin, sodium iodide symporter, thyroid-stimulating hormone receptor, and thyroperoxidase mRNA. Importantly, when embedded in collagen, these cells showed specific ^{125}iodide uptake activity, a hallmark of differentiated thyroid cells.

This study demonstrates that human thyroid nodule tissues contain a population of stem cells with SP phenotype and the capacity of clonal expansion in response to intense growth stimulation. These stem cells express typical gene profiles and are characterized by the ability of

self-renewal and differentiation into thyroid cells. Compared to thyrocytes, stem cells display a much higher proliferation rate upon acute growth stimulation which suggests a putative role of stem cells in the chronic growth factor-stimulated nodular transformation of the thyroid.

Further work is necessary to analyze how stem cell growth may potentially contribute to neoplastic thyroid growth that arises in nodular goiters and has to focus on the molecular and cellular aberrations that may occur on the long way from adult stem cells to differentiated thyroid cells *in vivo* and may be at the very beginning of thyroid tumorigenesis.

Zusammenfassung in deutscher Sprache

In den letzten Jahren sind adulte Stammzellen Gegenstand intensiver Forschung sowohl hinsichtlich ihrer physiologischen Bedeutung als auch in Hinblick auf ihre mögliche Bedeutung für die Entstehung und das Wachstum benigner und maligner Tumoren geworden. In den vorausgehenden Arbeiten unserer Arbeitsgruppe konnte zum ersten Mal der Nachweis von adulten Stammzellen in menschlichen Schilddrüsengeweben geführt werden. Bislang ist jedoch die Isolation von Schilddrüsenstammzellen aus dem Schilddrüsengewebe am Fehlen spezifischer Oberflächlichen-Antigenen gescheitert. Solche Antigenmarker sind für die Selektion und Isolierung intakter Zellen mittels FACS erforderlich. In der vorliegenden Arbeit sollten daher 1. die Anreichungs- und Isolierungs-Techniken für Schilddrüsenstammzellen etabliert werden, 2. die Bedingungen analysiert werden, unter denen die aus menschlichen Schilddrüsenknoten stammende, ruhenden (d.h. nicht proliferierenden) Stammzellen zum Wachstum gebracht werden können und 3. die Frage geklärt werden, ob diese Zellen das Potenzial zur Differenzierung in Schilddrüsenzellen beibehalten haben.

Die in dieser Arbeit angewandte Isolationstechnik von Schilddrüsenstammzellen aus menschlichen Schilddrüsenknoten mittels FACS basierte auf dem Nachweis der Expression von ABCG2-Membrantransportern. Aufgrund dieser typischen Genexpressionsprofile konnten eine Seitenpopulation (sogen. side population) von Stammzellen (SP-Zellen) von den übrigen Zellen der Schilddrüse (non-SP-Zellfraktion), bestehend aus endodermalen Vorläuferzellen und differenzierten Thyreozyten, differenziert werden. Morphologisch wiesen die SP-Zellen lichtmikroskopisch eine charakteristische Stammzellmorphologie.

Die Vermehrung und Differenzierung von isolierten Stammzellen wurde jedoch durch ihren Ruhezustand (sogen. quiescent state)und ihre verminderte Lebensfähigkeit in vitro begrenzt. Dieses Problem konnte durch neue Isolation- und Stimulationsmethoden überwunden werden. Nach intensiver Wachstumsstimulation in Co-Kultur mit normalen Schilddrüsenzellen und sogen. Nischenzellen wuchs eine kleine Zahl von Schilddrüsenstammzellen aus primären Zellkulturen heran und bildete dreidimensionale kugelförmige Klone. Diese nannten wir „thyro- spheres“. Diese „thyro-spheres“ bestanden aus sich stark vermehrenden Stamm- und Vorläuferzellen mit typischen Genexpressionsprofilen. Nach Induktion der Differenzierung durch Serum und TSH, wuchsen diese „thyro-spheres“-Zellen als Monolayer aus und ließen sich in Thyreozyten mit

typischen mRNA-Expressionsmustern (PAX8-, Thyreoglobulin-, Natrium-Jodid-Symporter-, TSH-Rezeptor- und Thyreoperoxydase- mRNA) differenzieren. Von besonderer Bedeutung war der Befund, dass nach Einbetten in Kollagen diese Zellen eine spezifische ^{125}Jodid-Aufnahme aufwiesen, was ein Kennzeichen für differenzierte Schilddrüsenzellen ist.

Diese Arbeit zeigt, dass menschliche Schilddrüsenknotengewebe Stammzellen enthalten, die einen SP-Phänotyp besitzen und ein Potenzial zur klonalen Expansion nach intensiver Wachstumsstimulation aufweisen. Diese Stammzellen exprimieren typische Genprofile und besitzen unter charakteristischen Kulturbedingungen ein Eigenvermehrungs- und Differen-zierungspotenzial. Im Vergleich zu Thyreozyten, weisen diese Stammzellen nach akuter Wachstumsstimulation eine erhöhte Proliferationsrate auf. Dies könnte auf eine mögliche Rolle von Stamm- bzw. Progenitorzellen bei der durch chronische Wachstumsstimulation induzierten, nodulären Transformation der Schilddrüse hindeuten.

Weitere Studien sind erforderlich, um die Bedeutung des Stammzellwachstums für die Entstehung neoplastischen Schilddrüsenwachstums zu analysieren. Zukünftige Forschung sollte dabei auf die molekularen und zellulären Aberrationen fokussieren, die während des langen Weges von adulten Stammzellen bis zur differenzierten Thyreozyten *in vivo* auftreten können und Ausgangspunkt der Schilddrüsentumorgenese sein könnten.

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