MURAJ
Minnesota Undergraduate Research & Academic Journal

MINNESOTA UNDERGRADUATE RESEARCH & ACADEMIC JOURNAL
VOLUME 8 ISSUE 1

C-Cell Hyperplasia in Cellular Reinoic Acid Binding Protein 1 (CRABP1) Knockout Male Mice Thyroid Gland

Jiaxuan Han[1] , Fatimah Najjar[2] , Li-Na Wei[2]

Abstract

The thyroid gland consists of follicles lined by thyrocytes, with parafollicular cells, including C-cells, residing between these follicles (Krinke & Weber, 2012). C-cell hyperplasia is the increase of C-cell proliferation which may progress to medullary thyroid carcinoma (MTC). MTC accounts for 4% of thyroid cancer (Kloos et al., 2009). Although thyrocytes originate from the endoderm and C-cells originate from the neural crest, several transcription factors are shared between these cell lineages (Cote et al., 2015). Cellular retinoic acid binding protein 1 (CRABP1) is highly expressed in the human thyroid gland (The Human Protein Atlas, 2021). Previously, CRABP1 was reported to play a crucial role in maintaining thyrocyte function. Knockout of CRABP1 (CKO) in mice has been shown to result in smaller thyroid follicles and an increased population of non-forming-follicles cells, observations that prompted the present study (Najjar et al., 2023). Here, we evaluated several transcription factors and histopathologic features of CKO mice. SOX17 and CALCA gene expression in 15-month-old mice are significantly higher in CKO mice than wild-type (WT). FOXA2 gene expression in 3-month-old mice is significantly higher in CKO than WT. Histopathological examination indicated significantly smaller follicle sizes and a greater number of cells in CKO mice compared with WT. These findings suggest a potential role for CRABP1 in maintaining thyrocytes and C-cell balance in the thyroid gland.

Keywords

C-cell hyperplasia, Thyroid gland, CRABP1

1 Introduction

The thyroid gland is a butterfly-shaped gland in the neck, which plays an important role in

[1] College of Liberal Arts, University of Minnesota, Twin Cities
[2] Department of Pharmacology, University of Minnesota Twin Cities

regulating metabolism, growth, and development. It secretes thyroid hormones, primarily triiodothyronine (T3) and thyroxine (T4). The thyroid gland is organized into follicles, which are filled with colloids. Colloids serve as a storage and production site for thyroid hormones. The thyroid follicles' walls are made up of many thyrocytes. These thyrocytes are the derivatives of the endoderm layer and are considered the functional unit of the thyroid gland. In between the thyroid follicles, there are C-cells, which are a type of parafollicular cells. C-cells originate from the neural crest and secrete a peptide hormone, calcitonin, that decreases the calcium level in the blood (Cote et al., 2015a; Kloos et al., 2009).

C-cell hyperplasia (CCH) is an increase in the number of C-cells in the thyroid gland, often accompanied by excessive calcitonin secretion. Under normal physiological conditions, CCH can be caused by chronic low circulating calcium, advancing age, and chronic thyroiditis; on the other hand, CCH can also result from a protooncogene mutation in RET that leads to thyroid carcinoma (Perry et al., n.d.). In a study by Guyétant et al in 1997, the prevalence of CCH was more than twice as high in male subjects as in females (Guyétant et al., 1997). Under normal conditions, C-cells occupy less than 0.1% of the total cell mass in the thyroid gland, concentrating in the middle and upper thirds of the lateral lobes. Under normal aging conditions, an age-dependent increase in C-cell number was shown in male subjects, whereas there was no obvious relationship between age and C-cell number in female subjects (Gibson et al., 1982).

CCH often leads to medullary thyroid carcinoma (MTC) (Guyétant et al., 2003). When CCH is caused by a RET protooncogene mutation, it carries an increased risk of developing MTC. Such condition is considered to be an undifferentiated thyroid carcinoma (Cote et al., 2015). According to American Thyroid Association (ATA) guidelines, CCH is classified as familial/ inherited or reactive/ physiological (Kloos et al., 2009). MTC develops from parafollicular cells and is characterized by the excess secretion of calcitonin. A blood calcitonin levels over 100 pg/ml is considered a positive prognostic value for MTC (Kiriakopoulos et al., 2022). MTC occurs in both sporadic and familial forms. According to the ATA, the 10-year disease-specific survival rate in MTC varies dramatically by stage: ~100% for stage I, ~93% for stage II, ~71% for stage III, and ~21% for stage IV disease. Many patients already present with regional lymph nodes metastases of the time of diagnosis (Kloos et al., 2009; Modigliani et al., 1998).

Several transcription factors are essential for the differentiation, maintenance, and functional regulation of thyroid tissue. Paired Box Gene 8 (PAX8) helps regulate development, differentiation, and maintenance of thyrocytes. NKX2-1 (TTF1) regulates thyrocyte differentiation and functions by driving thyroid-stimulating hormone receptor gene transcription. FOXA1 (TTF2) acts earlier in thyroid organogenesis and contributes to the structural maturation of thyroid follicles. SRY-box transcription factor 17 (SOX17) marks the endodermal identity. Forkhead Box A2 (FOXA2) indicates early thyroid/endodermal lineage integrity. Haematopoietically expressed homeobox (HHEX) marks the early thyroid progenitor activation or maintenance. In 2015 Johansson et al. demonstrated that FOXA1 and FOXA2 are co-expressed in both C-cell progenitors and differentiated C-cells, whereas FOXA2 expression is down-regulated in the thyrocytes lineage (Johansson et al., 2015). In contrast, thyrocyte differentiation requires the coordinated activity of

TTF1 and PAX8, while TTF1 alone is sufficient for C-cell differentiation (Nilsson & Williams, 2016).

Previous studies have shown that CRABP1 plays a crucial role in the development and maintenance of the thyroid gland. CKO mice have also shown phenotypes of primary hypothyroidism, where the thyroid gland fails to produce sufficient thyroid hormones, unlike secondary hypothyroidism, which originates from the central nervous system dysregulation of the thyroid gland. Under histopathological examination, CKO thyroid gland exhibits small follicles alongside cells that are not forming follicles (Najjar et al., 2023). In an observational study of several types of thyroid carcinomas, loss of CRABP1 was found in undifferentiated thyroid carcinomas like MTC (Celestino et al., 2018).

In this study, we evaluate the expression of thyrocytes and C-cell transcription factors through ages 3 months to 15 months. These key transcription factors determine the fate of progenitors to differentiate into thyrocytes or C-cells. Well-differentiated thyrocytes and C-Cells maintain PAX8 and FOXA2 expression, respectively, whereas loss of these transcription factors is linked to undifferentiated thyroid tumors and more aggressive phenotypes (López-Márquez et al., 2021; Pasca Di Magliano et al., 2000). Additionally, we assessed the morphological changes observed in 15 months old CKO male thyroid glands. CCH can be classified as focal or diffuse by histopathological examination. Focal C-cell clusters, less than five average follicular diameters, or similarly small-sized clusters of C cells scattered in interfollicular spaces in a specific location in the thyroid gland, on the other hand, diffuse CCH occurs in the whole thyroid gland. Unlike C-cell adenomas, CCH is not associated with compression of the follicular parenchyma.

2 Methods

2.1 Quantitative Real-Time PCR

RNA was extracted from thyroid tissue samples collected from both WT and CKO male mice. Tissues were homogenized in TRIzol (Thermo Fisher, Waltham, MA, USA, Cat#15596026), and the aqueous phase was collected to precipitate mRNA, and 2 mg of RNA was reverse transcribed to cDNA with a reverse transcript kit (Thermo Fisher, Cat#4368819). Quantitative real-time PCR (qPCR) was performed using the SYBR Green PCR Master Mix (Thermo Fisher, #K0253) on a real-time PCR machine (QuantStudio 3 Real-Time PCR system, Applied Biosystems Waltham, MA, USA). The qPCR reaction was performed in duplicate for each sample. The values were normalized to GAPDH and expressed as relative expression. Primers used are listed in Table 1.

Gene Name	Primer Sequence
GAPDH	Forward: 5′ -CAGAAGACTGTGGATGGCCCCTC-3′ ; Reverse: 5′ -CACGGAAGGCCATGCCAGTGAGC-3′
PAX8	Forward: 5′ -TGCTCAGCCTGGCAATGACAAC-3′ ; Reverse: 5′ -ACGAAGGTGCTTTCGAGGACCA-3′
TTF1	Forward: 5′ -CAGGACACCATGCGGAACAGC-3′ ; Reverse: 5′ -GCCATGTTCTTGCTCACGTCCC-3′
TTF2	Forward: 5′ -AACAGCATCCGCCACAACCTCA-3′ ; Reverse: 5′ -AGGAAGCTGCCGCTTTCGAACA-3′
HHEX	Forward: 5′ -CGGTCAAGTGAGGTTCTCCAAC-3′; Reverse: 5′ -CTCGGCGATTCTGAAACCAGGT-3′
SOX17	Forward: 5′ -GCCGATGAACGCCTTTATGGTG-3′; Reverse: 5′ -TCTCTGCCAAGGTCAACGCCTT-3′
FOXA2	Forward: 5′ -CGAGCACCATTACGCCTTCAAC-3′; Reverse: 5′ -AGTGCATGACCTGTTCGTAGGC-3′
CALCA	Forward: 5′ -GCACTGGTGCAGGACTATATGC-3′; Reverse: 5′ -CTCAGATTCCCACACCGCTTAG-3′
CRABP1	Forward: 5′ -ACCTGGAAGATGCGCAGCAGCGAG-3′ ; Reverse: 5′ -TAAACTCCTGCATTTGCGTCCGTCC-3′

Table 1. *Primer Sequences for qPCR.*

2.2 FIJI ImageJ

We harvested thyroid glands that were fixed in 10% formalin overnight and dehydrated in ethanol for paraffin block preparation. Tissue sections were cut at 5 µm and stained with hematoxylin and eosin stain for histopathological evaluation by a board-certified veterinary pathologist. We observed the tissue sections using a LEICA DMIRB microscope equipped with a 20x objective, and representative fields were captured for analysis. We conducted all image analyses manually in FIJI (ImageJ), with the scale of 3.6140 pixels/µm.

2.3 Statistical analysis

In qPCR experiment, 4 mice were used for 3-month-old WT, 5 mice were used for 3-month-old CKO; 4 mice were used for 6-month-old WT, 4 mice were used for 6-month-old CKO; 6 mice were used for 9-month-old WT, 11 mice were used for 9-month-old CKO; 7 mice were used for 15-month-old WT, 5 mice were used for 15-month-old CKO. T-test was performed to compare groups. In the histopathology analysis, 3 animals per group were used, and 12 sections were randomly selected to analyze the area of interest, and the data represents the average of follicle size and total number of cells. A t-test was performed.

3 Results

3.1 Undifferentiated gene expression increased in the CKO thyroid gland

Most of the transcription factors that regulate thyrocytes and C-cell differentiation displayed a similar growing pattern in both WT and CKO mice with advancing age. The FOXA2 gene expression of 3-month-old mice was significantly higher in CKO than WT as shown in Figure 1A. The upregulation of FOXA2 observed is a hallmark for CCH. The SOX17 gene expression of 15 -month-old mice was significantly higher in CKO than that of WT as shown in Figure 1A. Increased expression of SOX17 is a hallmark of stemness in cells, preventing precursor cells from differentiating into thyrocytes or C-cell progenitors. This suggests that thyrocytes and C-cells were shifted to a more immature and unspecialized state. Lastly, calcitonin (CALCA) gene expression increased significantly in 15-month-old CKO mice (Figure 1B). These findings suggest that CKO mice developed C-cell hyperplasia at the age of 15 months.

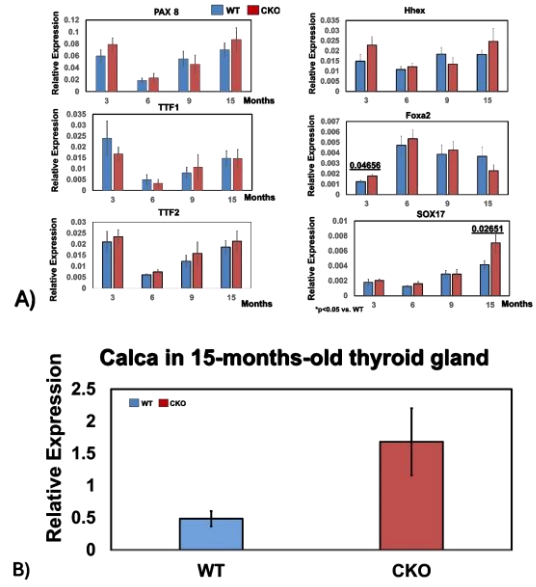

Figure 1. *(A) Quantitative real-time PCR (qPCR) performed for CKO and WT of 3 months, 6 months, 9 months and 15 months old male mice for gene PAX8, TTF1, TTF2, FOXA2, HHEX and SOX17. Values are Mean ± SD/ n= 4-11 Mice /group. Significant increase of FOXA2 expression in 3-month-old CKO mice compared to WT at the same age. [t (9) =5.122, p=0.0018, df=6.444]. Significant increase of SOX17 expression in 15 -month-old CKO mice compared to WT at the same age. [t (12) = 4.479, p=0.0067, df=4.937] (B) Thyroid Gland CALCA expression in 15-month-old mice of WT and CKO. CALCA tends to be expressed significantly higher in CKO than WT. [t (6) =3.882, p=0.0515, df=2.205].*

3.2 Histopathology of CKO thyroid gland

In the thyroid glands of aged WT male mice, follicles appear well-differentiated and exhibit mature functions in thyroid hormone synthesis and storage. In Figure 2A, the follicles of 15-month-old WT mice are shown to be large, lined with small number of undifferentiated cells (red arrows). Whereas in 15-month-old CKO mice, the follicles appeared to be smaller, surrounded by larger number of undifferentiated cells (green arrows). Such conditions are also supported statistically. As shown in Figure 2B, the follicle sizes of 15-month-old WT are significantly larger than those of CKO. The cell number of 15-month-old WT is significantly smaller than that of CKO.

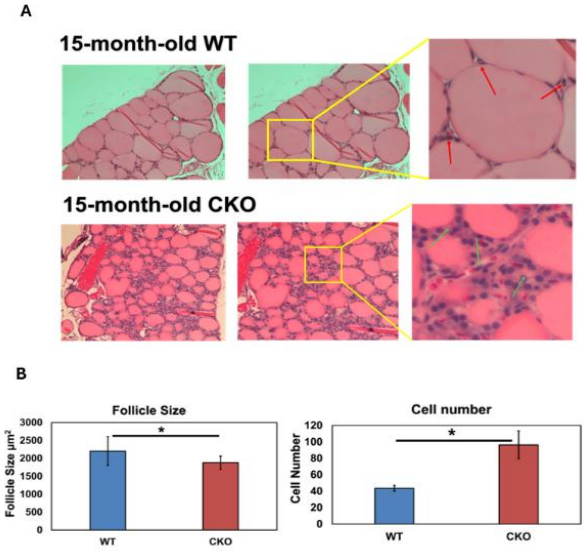

Figure 2. *(A) Hematoxylin and eosin (H&E) - stained thyroid gland from CKO and WT of 15-month-old male mice. (B) Thyroid gland follicle and cell number of WT and CKO male mice at the age of 15 months old. Values are Mean ± SD/ n=3 Mice /group. Significant changes in CKO follicle sizes compared to WT at the same age. [t (122) =6.099, p<0.0001, df=117.3]. Significant changes in CKO cell number were observed compared to WT at the same age. [t (260) =30.1, p<0.0001, df=99.56]. *p<0.05 considered scientific significant vs WT.*

3.3 CRABP1 expression in aging thyroid gland

Finally, we assessed CRABP1 expression at different ages, as shown in Figure 3, and found that CRABP1 expression increased with age, emphasizing that CRABP1 may have a critical role in maintaining the homeostasis of thyrocytes and C-cells.

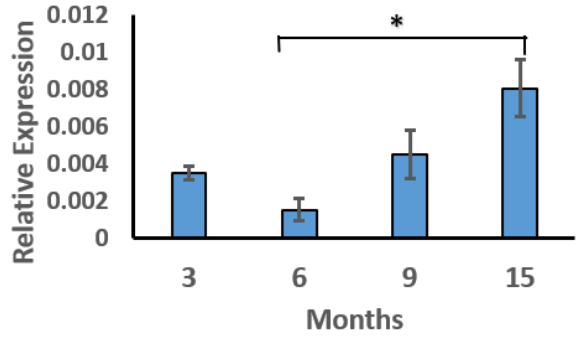

Figure 3. *CRABP1 gene expression increased significantly through 6 months old to 15-month-old in WT mice. [t(9)=8.731, p<0.01, df=5.436] *p<0.05 considered statistical significance.*

4 Conclusion

The present study demonstrates that CKO thyroid glands might exhibit a transcription factor pattern mimicking CCH. FOXA2 expression increased at an early stage, while increases in SOX17 and CALCA occurred at an advanced age. In addition, reduced follicle size and increased cell number in the CKO thyroid gland were observed. These findings suggest that lack of CRABP1 expression alters the transcription factor network, leading to CCH-like conditions as well as follicle degeneration. This ultimately causes inadequate thyrocyte and C-cell differentiation. Further investigation is required to understand the

specific mechanisms by which CRABP1 affects C-cell differentiation, causing CCH.

It is important to distinguish C-cell hyperplasia from C-cell hypertrophy. High activity of C-cells, or hypertrophy, refers to increased calcitonin release without an increase in cell number, whereas C-cell hyperplasia is defined by an increased number of C-cells with unchanged activity per cell. The morphological and transcriptional changes observed in CKO mice are consistent with hyperplasia rather than hypertrophy, indicating that CRABP1 deficiency increases C-cell number rather than increases C-cell size.

TTF1, TTF2, and PAX8 gene expression patterns remain the same in both WT and CKO. FOXA2 gene expression decreases with advancing age in WT and, while CKO thyroid gland exhibited a greater decrease in FOXA2 expression, this decrease was not significant. Increase of FOXA2 at an early age (3 months) induces C-cell population, followed by a decrease in FOXA2 and an increase in SOX17 expression. This suggests that CKO thyroid gland progression to CCH could lead to MTC.

One limitation of this study is that the samples used for evaluation gene expressions of transcription factors were withdrawn from the whole thyroid tissue, including both thyrocytes and C-cells. This cellular heterogeneity leads to increased variability and limits reliability to assess age-related transcriptional change. Future investigations may employ isolated thyrocytes and C-cells to more precisely investigate CRABP1 regulation in thyroid function.

Acknowledgements

This project was supported by the University of Minnesota's Office of Undergraduate Research.

C-Cell Hyperplasia in Cellular Retinoic Acid Binding Protein 1 (CRABP1) Knockout Male Mice Thyroid Gland

References

Celestino, R., Nome, T., Pestana, A., Hoff, A. M., Gonçalves, A. P., Pereira, L., Cavadas, B., Eloy, C., Bjøro, T., Sobrinho-Simões, M., Skotheim, R. I., & Soares, P. (n.d.). *CRABP1, C1QL1 and LCN2 are biomarkers of differentiated thyroid carcinoma, and predict extrathyroidal extension*. https://doi.org/10.1186/s12885-017-3948-3

Cote, G. J., Grubbs, E. G., & Hofmann, M. C. (2015). Thyroid C-Cell Biology and Oncogenic Transformation. *Recent Results in Cancer Research*, *204*, 1–39. https://doi.org/10.1007/978-3-319-22542-5_1

CRABP1 protein expression summary - The Human Protein Atlas. (n.d.). Retrieved March 14, 2026, from https://www.proteinatlas.org/ENSG00000166426-CRABP1

Gibson, W. G. H., Peng, T.-C., & Croker, B. P. (n.d.). *Age-Associated C-Cell Hyperplasia in the Human Thyroid*.

Guyétant, S., Josselin, N., Savagner, F., Rohmer, V., Michalak, S., & Saint-André, J.-P. (2003). *C-cell hyperplasia and medullary thyroid carcinoma: Clinicopathological and genetic correlations in 66 consecutive patients*. *Modern Pathology*, *16*(8), 756–763. https://doi.org/10.1097/01.MP.0000081727.75778.0C

Guyétant, S., Rousselet, M.-C., Durigon, M., Chappard, D., Franc, B., Guerin, O., & Saint-André, J.-P. (1997). Sex-Related C Cell Hyperplasia in the Normal Human Thyroid: A Quantitative Autopsy Study. *The Journal of Clinical Endocrinology & Metabolism*, *82*(1), 42–47. https://doi.org/10.1210/jcem.82.1.3684

Johansson, E., Andersson, L., Örnros, J., Carlsson, T., Ingeson-Carlsson, C., Liang, S., Dahlberg, J., Jansson, S., Parrillo, L., Zoppoli, P., Barila, G. O., Altschuler, D. L., Padula, D., Lickert, H., Fagman, H., & Nilsson, M. (2015). Revising the embryonic origin of thyroid C cells in mice and humans. *Development (Cambridge)*, *142*(20), 3519–3528. https://doi.org/10.1242/dev.126581

Kiriakopoulos, A., Giannakis, P., & Menenakos, E. (2022). Calcitonin: current concepts and differential diagnosis. *Therapeutic Advances in Endocrinology and Metabolism*, *13*. https://doi.org/10.1177/20420188221099344

Kloos, R. T., Eng, C., Evans, D. B., Francis, G. L., Gagel, R. F., Gharib, H., Moley, J. F., Pacini, F., Ringel, M. D., Schlumberger, M., & Wells, S. A. (2009). Medullary thyroid cancer: management guidelines of the American Thyroid Association. *Thyroid : Official Journal of the American Thyroid Association*, *19*(6), 565–612. https://doi.org/10.1089/thy.2008.0403

Krinke, G. J., & Weber, K. (2012). Histology. *The Laboratory Mouse*, *38*(6), 161–192. https://doi.org/10.1016/B978-0-12-382008-2.00008-8

López-Márquez, A., Carrasco-López, C., Fernández-Méndez, C., & Santisteban, P. (2021). Unraveling the Complex Interplay Between Transcription Factors and Signaling Molecules in Thyroid Differentiation and Function, From Embryos to Adults. *Frontiers in Endocrinology*, *12*, 654569. https://doi.org/10.3389/fendo.2021.654569

Modigliani, E., Cohen, R., Campos, J. M., Conte-Devolx, B., Maes, B., Boneu, A., Schlumberger, M., Bigorgne, J. C., Dumontier, P., Leclerc, L., Corcuff, B., & Guilhem, I. (1998). Prognostic factors for survival and for biochemical cure in medullary thyroid carcinoma: Results in 899 patients. *Clinical Endocrinology*, *48*(3), 265–273. https://doi.org/10.1046/j.1365-2265.1998.00392.x

Najjar, F., Nhieu, J., Wei, C. W., Milbauer, L., Burmeister, L., Seelig, D., & Wei, L. N. (2023). Deleting Cellular Retinoic-Acid-Binding Protein-1 (Crabp1) G CausesAdult-Onset Primary Hypothyroidism in Mice. *Endocrines 2023, Vol. 4, Pages 138-150*, *4*(1), 138–150. https://doi.org/10.3390/endocrines4010013

Nilsson, M., & Williams, D. (2016). On the Origin of Cells and Derivation of Thyroid Cancer: C Cell Story Revisited. *European Thyroid Journal*, *5*(2), 79–93. https://doi.org/10.1159/000447333

Pasca Di Magliano, M., Di Lauro, R., Zannini, M., & Rosenfeld, M. G. (n.d.). *Pax8 has a key role in thyroid cell differentiation*. Retrieved March 18, 2026, from www.pnas.orgcgi-doi10.1073pnas.240336397

Perry, A., Molberg, K., & Albores-Saavedra, J. (1996). *Physiologic Versus Neoplastic C-Cell Hyperplasia of the Thyroid Separation of Distinct Histologic and Biologic Entities*. https://doi.org/10.1002/(SICI)1097-0142(19960215)77:4<750::AID-CNCR22>3.0.CO;2-Z