Aus dem Anatomischen Institut

der medizinischen Fakultät

der Universität des Saarlandes, Homburg/Saar

Prof. Dr. med. Thomas Tschernig

Immunhistochemischer Nachweis der nichtselektiven Kationenkanäle TRPC3 und TRPC6 in endokrinen Drüsen des Menschen

Kumulative Dissertation zur Erlangung des Grades eines Doktors der

Medizin der medizinischen Fakultät der

UNIVERSITÄT DES SAARLANDES

2025

vorgelegt von Emilie Charlotte Kirstein

geboren am 06.12.2000 in Münster

Tag der Promotion: 12.01.2026
Dekan: Univ.-Prof. Dr. med. dent. Matthias Hannig
1. Berichterstatter: Prof. Dr. med. Thomas Tschernig
2. Berichterstatter: Univ. Prof. Dr. rer. nat. Ulrich Boehm

Inhaltsverzeichnis

Summary

The regulation of Ca^{2+}-signaling plays a crucial role in a vast number of physiological and pathophysiological processes. Especially, Transient Receptor Potential Canonical (TRPC) channels, including TRPC3 and TRPC6, are integral parts of these signaling pathways and may show great new potential as targets for novel therapeutic approaches. Their ability to modulate intracellular Ca^{2+}-levels makes them key factors in the regulation of cellular functions such as proliferation, migration and secretion, but also in the development and progression of diseases such as cancer, endocrine disorders and inflammatory diseases.

The herein presented studies investigated TRPC3/6 expression in human parathyroid, thyroid, and pancreatic tissues using immunohistochemistry with knockout-validated antibodies. Tissue samples were obtained from surgical procedures and body donations. A software-based scoring system was used to quantify staining intensity where applicable. Altogether, they provide new insights into the protein expression patterns of TRPC channels and their potential relevance in endocrine tissues, aiming to improve our understanding of their tissue-specific distribution.

The first study investigates the expression patterns of TRPC3/6 channels both in healthy and in primary hyperparathyroidism-diseased parathyroid tissue. Protein expression of TRPC3/6 was detected in the human parathyroid gland, particularly in chief and oxyphilic cells. It was interesting to observe that the TRPC3 staining score was statistically significantly lower in diseased tissue than in healthy tissue.

The second study analyzed the distribution of TRPC3/6 channels in the healthy human thyroid gland. Immunostaining of TRPC3/6 in thyrocytes revealed irregular patterns with some cells showing intense staining and others exhibiting no staining. Comparison of calcitonin and TRPC3/6 immunostained sections strongly indicated expression of TRPC3/6 in C-cells.

The third study focuses on the immunohistochemical analysis of TRPC3/6 in endocrine and exocrine pancreatic tissue. TRPC3/6 proteins were detected in various structures of the pancreas, including acinar cells and epithelial cells of the ductal, intralobular and interlobular ducts. Endocrine islets of Langerhans were homogeneously labeled by anti-TRPC3 and anti-TRPC6 antibodies.

These findings enhance the understanding of TRPC3/6 distribution and function in selected endocrine tissues.

Zusammenfassung

Die Regulation von Ca^{2+}-Signalen spielt eine entscheidende Rolle in einer Vielzahl physiologischer und pathophysiologischer Prozesse. Insbesondere die kanonischen transienten Rezeptor-Potential (TRPC)-Kanäle, darunter TRPC3 und TRPC6, sind integrale Bestandteile dieser Signalwege und zeigen ein großes Potenzial als Zielstrukturen für neue therapeutische Ansätze. Ihre Fähigkeit, intrazelluläre Ca^{2+}-Spiegel zu modulieren, macht sie zu Schlüsselfaktoren in der Regulation von Zellfunktionen wie Proliferation, Migration und Sekretion, aber auch in der Entwicklung und Progression von Erkrankungen wie Krebs, endokrinen Störungen und Entzündungserkrankungen.

In den hier vorgestellten Studien wurde die Expression von TRPC3/6 in menschlichem Nebenschilddrüsen-, Schilddrüsen- und Pankreasgewebe mittels Immunhistochemie mit Knockout-validierten Antikörpern untersucht. Die Gewebeproben stammten aus chirurgischen Eingriffen und Körperspenden. Zur Quantifizierung der Färbeintensität wurde, wenn anwendbar, ein softwaregestütztes Scoring-System verwendet. Zusammenfassend liefern die hier präsentierten Studien neue Erkenntnisse bezüglich der Proteinexpressionsmuster von TRPC-Kanälen und ihrer potenziellen Relevanz in endokrinen Geweben und sollen unser Verständnis ihrer gewebespezifischen Verteilung verbessern.

Die erste Studie untersucht die Expressionsmuster von TRPC3/6-Kanälen in gesundem sowie mit primärem Hyperparathyreoidismus erkranktem Nebenschilddrüsengewebe. Es konnte die Proteinexpression von TRPC3/6 in der menschlichen Nebenschilddrüse nachgewiesen werden, insbesondere in Haupt- und oxyphilen Zellen. Auffällig war, dass der TRPC3-Färbescore in erkranktem Gewebe statistisch signifikant niedriger war als in gesundem Gewebe.

Die zweite Studie analysiert die Verteilung der TRPC3/6-Kanäle in der gesunden menschlichen Schilddrüse. Die Immunfärbung von TRPC3/6 in Thyreozyten zeigte unregelmäßige Muster, bei denen einige Zellen eine intensive Färbung aufwiesen, während andere keine Färbung zeigten. Der Vergleich von Calcitonin- und TRPC3/6-immungefärbten Schnitten deutete stark auf eine Expression von TRPC3/6 in C-Zellen hin.

Die dritte Studie fokussiert sich auf die immunhistochemischen Untersuchungen von TRPC3/6 im endokrinen und exokrinen Pankreasgewebe. Die TRPC3/6-Proteine konnten in verschiedenen Strukturen des Pankreas nachgewiesen werden, darunter Azinus-Zellen sowie Epithelzellen der Schalt-, intralobulären und interlobulären Gänge. Endokrine Langerhans-Inseln wurden eindeutig und homogen durch Anti-TRPC3- und Anti-TRPC6-Antikörper markiert.

Die Ergebnisse tragen zu einem besseren Verständnis der Verteilung und Funktion von TRPC3/6 in ausgewählten endokrinen Geweben bei.

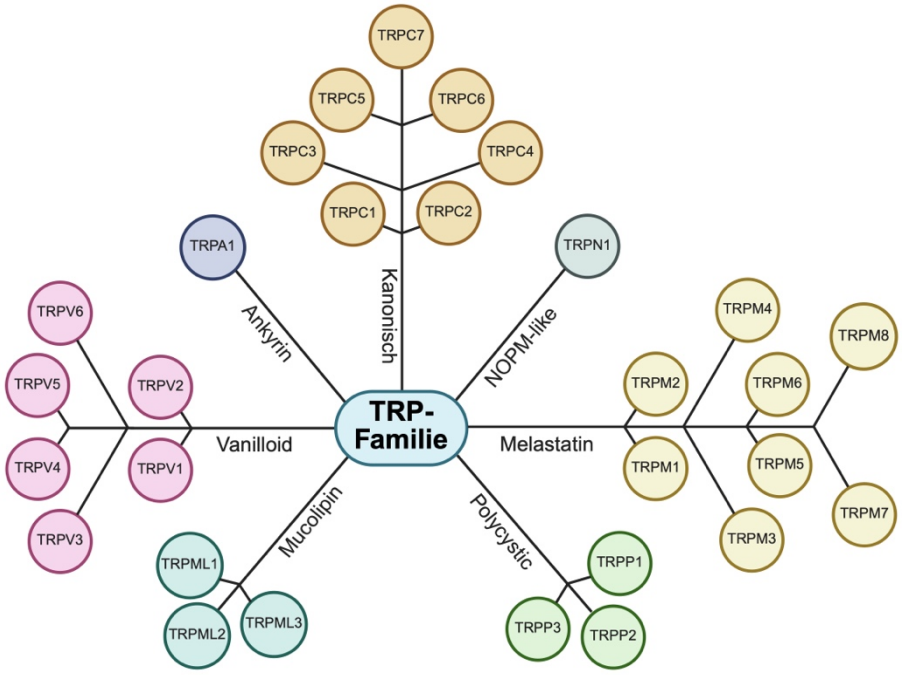
1. Einleitung und Motivation

1.1 Hintergrund und Relevanz von TRPC-Kanälen

Die Familie der transienten Rezeptor-Potenzial-Kanäle (TRP-Kanäle) stellt eine heterogene Gruppe von Ionenkanälen dar, die die Funktion zellulärer Sensoren ausüben und auf diverse physikalische und chemische Reize reagieren. Sie sind für ihre Rolle in verschiedensten sensorischen Prozessen bekannt, wie der Wahrnehmung von Temperatur, Schmerz, Geruch und mechanischen Reizen.[100] Erstmalig wurden sie 1969 in Forschungen an der Fruchtfliege *Drosophila melanogaster* beschrieben, die eine Mutante identifizieren konnten, deren Photorezeptoren auf Lichtreize nur transient mit einem Potential reagierten.[80] Der Begriff „transient receptor potential" (TRP) leitet sich hier her ab.[74] In der Folge gelang es, die Sequenz des dafür verantwortlichen TRP-Proteins zu bestimmen und es zeigte sich, dass es sich bei dem TRP-Protein um einen Ca^{2+}-permeablen Ionenkanal in den Rezeptoren von *Drosophila melanogaster* handelt. Dies führte zu einer Identifizierung einer ganzen Familie von TRP-Kanälen, darunter die kanonischen (TRPC), die Melastatin- (TRPM), die Vanilloid- (TRPV), die Mucolipin- (TRPML), die Polycystin- (TRPP), die Ankyrin- (TRPA) und die „no mechanoreceptor potential C" (TRPN)-Unterfamilien (s. Abbildung 1).[74,80] Sie kommen zahlreich in Organismen, einschließlich des Menschen, vor und spielen eine zentrale Rolle in einer Vielzahl physiologischer und pathophysiologischer Prozesse.[28,74,100] Jeder Unterfamilie sind spezifische biochemische Aktivierungsmechanismen und physiologische Prozesse zugeordnet, was die Vielfalt dieser TRP-Kanal-Superfamilie unterstreicht.[84] Allen Gruppen der TRP-Kanal-Superfamilie ist die Ausbildung einer Kanalpore zwischen der fünften und sechsten Transmembrandomäne gemein.[100] Im Fokus dieser Arbeit steht die Unterfamilie der TRPC-Kanäle. Als Ca^{2+}-permeable, nichtselektive Kationenkanäle[1] ermöglichen sie den Einstrom von Ca^{2+} und anderen Kationen und tragen somit entscheidend zur zellulären Ca^{2+}-Homöostase und -Signalweiterleitung bei.[82]

Abbildung 1: **Familie der TRP-Kanäle.**

Die Abbildung stellt eine strukturelle Darstellung der sieben Hauptfamilien der TRP-Kanäle dar. Jede Unterfamilie ist weiter in Subgruppen unterteilt, die die spezifischen Mitglieder der jeweiligen Familie aufführen. (*Darstellung auf folgender Seite*)



Struktur und Funktion der TRPC-Kanäle

Die TRPC-Unterfamilie umfasst sieben Mitglieder (TRPC1-7), die sich in ihrer Gewebeverteilung und Funktion unterscheiden.[1] Die Bezeichnung „c“ steht für „kanonisch“, beziehungsweise „klassisch“, in Anlehnung an ihre Ähnlichkeit zu den entdecken Kanälen in der *Drosophila melanogaster*. Die TRPC-Unterfamilie wird in die Subgruppen TRPC1, TRPC2, TRPC 3/6/7 und TRPC 4/5 untergliedert.[1,76] In humanem Gewebe kommen sechs der sieben Subtypen vor. TRPC2 liegt hier als Pseudogen vor und codiert für kein funktionsfähiges Protein.[69] TRPC-Kanäle sind nicht-selektive Kationenkanäle mit unterschiedlich ausgeprägter Ca^{2+}-Permeabilität.[101] Strukturell sind TRPC-Kanäle als Tetramere aufgebaut, wobei jedes Monomer aus sechs Transmembran-Domänen, den alpha-Helices, besteht (s. Abbildung 2).[55] Die ersten vier transmembranen alpha-Helices (S1-S4) bilden eine spannungssensorähnliche Domäne. Die Ca^{2+}-Permeabilität wird durch eine Pore gewährleistet, die mitunter von der fünften und sechsten Transmembran-Domäne (S5-S6) gebildet wird.[101] Die sieben TRPC-Kanäle weisen eine konservierte Sequenz im C-terminalen Bereich auf. Darüber hinaus besitzen sie 3-4 ankyrinartige Wiederholungen am NH2-terminalen Ende.[101] Wichtig zu erwähnen ist, dass TRPC3 und TRPC6, zusammen mit TRPC7, eine eng verwandte Unterfamilie bilden, die sich durch eine hohe Aminosäureidentität von 70–80 %[99] sowie gemeinsame funktionelle, regulatorische und pharmakologische Eigenschaften auszeichnet. Ein einzigartiges Merkmal der TRPC3- und TRPC6-Kanäle ist ihre verlängerte extrazelluläre S3-Helix[95], die eine potenzielle Rolle in der Adressierung kleiner Moleküle spielen könnte.[101] Zudem erlaubt ihre Architektur eine feine Regulation der Kanäle durch unterschiedliche Stimuli, sei es durch rezeptor- oder speicherbetriebene Mechanismen.[1,34]

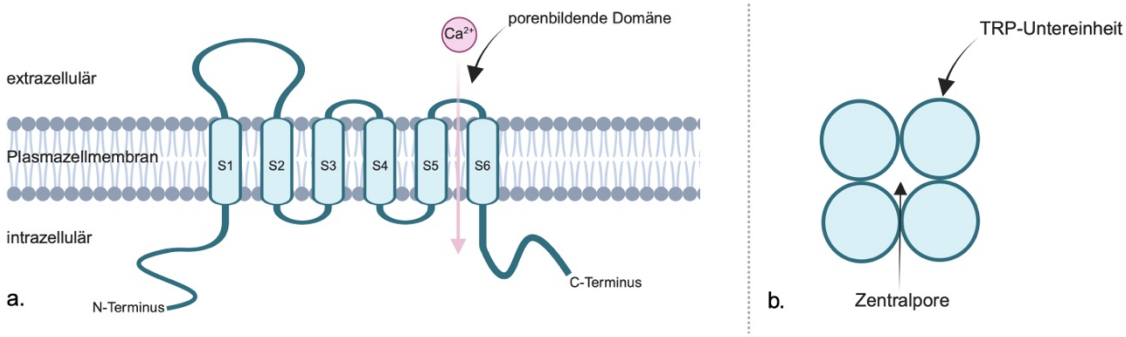


Abbildung 2: **Das TRPC-Protein.**

a: Schematische Darstellung der Transmembran-Domänen des TRPC-Kanals. Der Kanal besteht aus sechs transmembranen Helices (S1-S6), wobei die fünfte und sechste die Pore des Kanals bilden. Die Länge und Aminosäuresequenz der C- oder N-Termini variiert je nach TRPC-Unterform.
b: Tetramere Struktur des TRPC-Kanals. Der funktionelle Kanal wird durch die Assemblierung von vier Untereinheiten gebildet, die symmetrisch um eine zentrale Pore angeordnet sind.

TRPC-Kanäle und Ca^{2+}-Signalwege

Die TRPC-Kanäle werden durch komplexe Signalkaskaden aktiviert, die die Phospholipase C (PLC) sowie deren sekundäre Botenstoffe Inositoltriphosphat (IP_3) und Diacylglycerol (DAG) umfassen (s. Abbildung 3). Während DAG einige in der Plasmamembran liegende TRPC-Kanäle direkt aktiviert und dadurch rezeptorgesteuerte Ca^{2+}-Einstromwege vermittelt, sorgt IP_3 für eine Entleerung intrazellulärer Ca^{2+}-Speicher, insbesondere des endoplasmatischen Retikulums (ER). Dies führt zur Aktivierung speicherbetriebene Kanäle. Eine Tetramerisierung des Ca^{2+}-Sensors „stromal interacting molecule 1“ (STIM1) im ER, bei erschöpften Ca^{2+} Speicher, begünstigt die Stimulation von ORAI-Kanälen (calcium release-activated calcium channels) in der Plasmamembran, sowie von Mitgliedern der TRP-Familie, insbesondere der TRPC-Unterfamilie.[45,46,96] Beide Signalwege – sowohl die rezeptorgesteuerten als auch die speicherbetriebene Ca^{2+}-Einstromwege – tragen zu einem schnellen und transienten Anstieg der freien zytosolischen Ca^{2+}-Konzentration bei.

Diese dualen Aktivierungsmechanismen illustrieren die Vielseitigkeit der TRPC-Kanäle in der Regulation intrazellulärer Ca^{2+}-Konzentrationen. Ca^{2+} ist als universeller sekundärer Botenstoff an der Koordination zahlreiche zelluläre Prozesse wie Exozytose, Genexpression und Zellproliferation beteiligt.[12,79,80]

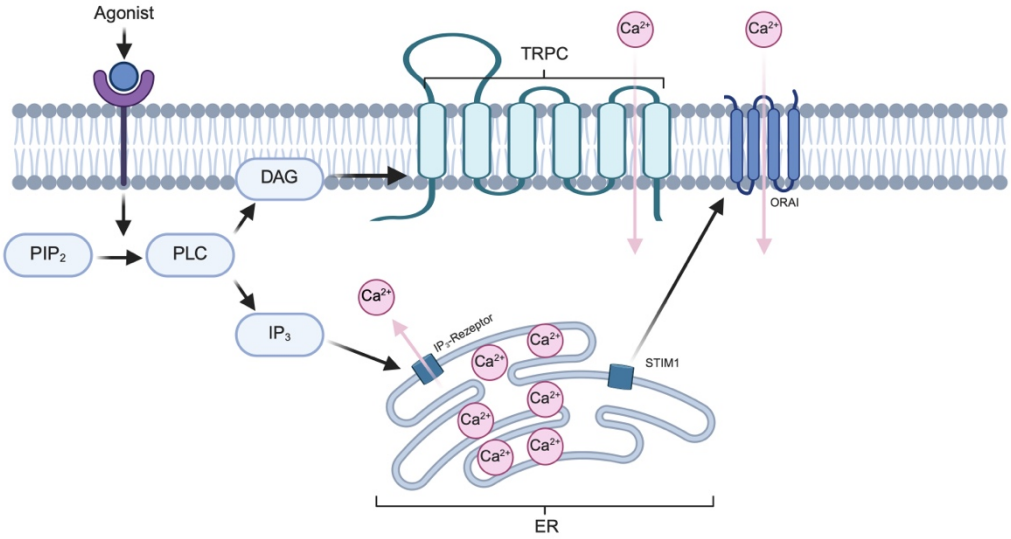


Abbildung 3: **Die Ca^{2+}-Signaltransduktion.**
Die Abbildung veranschaulicht die Aktivierung der TRPC-Kanäle durch Signalkaskaden, die die Phospholipase C (PLC) aktivieren. Die hydrolytische Spaltung von Phosphatidylinositol-4,5-bisphosphat (PIP_2) durch PLC führt zur Bildung von Inositol-1,4,5-trisphosphat (IP_3) und Diacylglycerol (DAG). DAG aktiviert direkt TRPC3 und TRPC6, was einen Ca^{2+}-Einstrom aus dem extrazellulären Raum bewirkt. IP_3 bindet an IP_3-Rezeptoren (IP_3R) im endoplasmatischen Retikulum (ER) und löst die Freisetzung von gespeicherten Ca^{2+} aus. Die Erschöpfung der Ca^{2+}-Speicher wird durch das „Stromal Interaction Molecule 1“ (STIM1) detektiert, was daraufhin Orai-Kanäle in der Plasmamembran aktiviert.

Innerhalb der TRPC-Kanäle haben insbesondere auch TRPC3 und TRPC6 aufgrund ihrer zentralen Rolle bei der Ca^{2+}-Signaltransduktion wissenschaftliche Aufmerksamkeit erfahren. Diese Kanäle neigen zur Bildung heteromerer Komplexe[4,44,54], was ihr funktionelles Repertoire erweitert und differenzierte Antworten auf physiologische Anforderungen ermöglicht. Zusammen mit TRPC7 gehören TRPC3 und TRPC6 zu den TRPC-Kanälen, die durch Diacylglycerol (DAG) und damit auf diese Weise direkt aktiviert werden können.[34]

Physiologische und pathophysiologische Bedeutung

Die Verteilung und Relevanz der TRPC-Kanäle erstreckt sich auf eine Vielzahl von Organsystemen. Ihre Rolle bei der Sinneswahrnehmung[56], der renalen Ca^{2+}-Resorption[31], der kardiovaskulären[75] und der pulmonalen[97] Regulation verdeutlicht ihre physiologische Bedeutung. Darüber hinaus wird eine Dysregulation der TRPC-Kanäle mit pathologischen Zuständen wie kardialer Hypertrophie, Onkogenese und neurodegenerativen Erkrankungen in Verbindung gebracht.[55] Da sich diese Untersuchung spezifisch auf die TRPC3 und TRPC6 Kanäle der TRP-Superfamilie konzentriert, soll im Folgenden ein kurzer Überblick

über das Vorkommen und die Bedeutung dieser beiden im Zusammenhang mit physiologischen und pathophysiologischen Prozessen gegeben werden.

TRPC3 weist eine breite gewebespezifische Verteilung auf und ist in zahlreichen Organen und Zelltypen exprimiert, darunter Gehirn[39], Herz[102], Lunge[68], Nieren[21], Nerven-[33] sowie im Immunsystem[2]. Besonders ausgeprägt ist seine Expression in den Purkinje-Zellen des Kleinhirns sowie im Hypothalamus.[41,85] Auch in myokardialem und glattem Muskelgewebe lässt sich TRPC3 nachweisen.[36,37] Funktionell ist TRPC3 eng mit der zellulären Ca^{2+}-Homöostase verknüpft. Es ermöglicht den Ca^{2+}-Einstrom in die Zelle und übernimmt dadurch eine zentrale Rolle bei Prozessen wie Muskelkontraktion, neuronaler Signalübertragung, Zellproliferation und synaptische Plastizität – insbesondere im zentralen Nervensystem.[19,40] In der Niere ist TRPC3 vor allem in den proximalen und distalen Tubuli sowie im Sammelrohr lokalisiert und trägt dort zur transzellulären Ca^{2+}-Reabsorption und luminalen Ca^{2+}-Homöostase bei.[21,31] Aus pathophysiologischer Sicht spielt TRPC3 eine wesentliche Rolle bei der Entstehung und Progression kardiovaskulärer und neurodegenerativer Erkrankungen.[26] Bei kardialer Hypertrophie vermittelt TRPC3 über den verstärkten Ca^{2+}-Einstrom die Aktivierung des Transkriptionsfaktors *nuclear factor of activated T-cells* (NFAT), der für die Expression hypertrophieassoziierter Gene verantwortlich ist. In Tiermodellen konnte gezeigt werden, dass die genetische Deletion von TRPC3 eine protektive Wirkung gegen solche Umbauprozesse hat.[77,88] Auch in der Niere deuten veränderte TRPC3-Expressionmuster bei Hyperkalzurie und Nephrokalzinose auf eine pathophysiologische relevante Rolle hin, wobei eine verminderte Kanalaktivität mit einer gestörten Ca^{2+}-Resorption in Zusammenhang gebracht wird.[29] Zur therapeutischen Modulation von TRPC3 wurde der selektive Antagonist Pyr3 entwickelt. In präklinischen Studien wies dieser vielversprechende Effekte bei der Behandlung von hypertrophen Herzveränderungen und neurodegenerativen Erkrankungen auf.[17,65]

TRPC6 ist vor allem in Plazenta, Herz, Lunge, Niere und im Gehirn exprimiert.[18,22] Innerhalb der Niere kommt TRPC6 vermehrt in den Glomeruli aber auch im Tubulussystem vor[32], während es im zentralen Nervensystem vor allem im Hippocampus und in der Großhirnrinde exprimiert wird.[91,106] Physiologisch übernimmt TRPC6 eine zentrale Rolle in der Regulation des vaskulären Tonus und der glomerulären Filtration. Wie TRPC3 vermittelt es den Ca^{2+}-Einstrom in die Zellen und beeinflusst dadurch vielfältige zelluläre Funktionen.[23,25] Pathologisch ist TRPC6 unter anderem an der Entstehung pulmonaler Hypertonie beteiligt. Seine Überexpression in glatten Gefäßmuskelzellen führt zu verstärktem Ca^{2+}-Einstrom, was eine übermäßige Vasokonstriktion zur Folge hat.[105] In der Niere führen Mutationen im TRPC6-Gen zu einer Überaktivierung des Kanals, was die Integrität des podozytären Zytoskeletts beeinträchtigt und eine familiäre fokale segmentale Glomerulosklerose (FSGS)

begünstigt.[72] Darüber hinaus wird TRPC6 bei verschiedenen Tumorarten überexprimiert und fördert dort die Zellproliferation, Migration und Angiogenese.[8] Zur gezielten Hemmung von TRPC6 wurde der Antagonist SAR7334 entwickelt, der in Tiermodellen bereits vielversprechende Effekte bei der Behandlung von Bluthochdruck und glomerulären Erkrankungen zeigte.[66]

Aufgrund ihrer breiten Beteiligung an krankheitsrelevanten Signalwegen stellt sich die Frage, ob und inwieweit TRPC3 und TRPC6 auch in hormonbildenden Organen eine Rolle spielen. Da gerade in endokrinen Geweben Ca^{2+}-Signalen eine Schlüsselrolle für die Hormonsekretion und Zellfunktion zukommt[20,38], ist das Verständnis der TRPC-vermittelten Mechanismen entscheidend, um potenzielle Einflüsse auf endokrine Regulation und pathologische Veränderungen dieser Organe besser zu verstehen. In hormonproduzierenden Geweben wie der Hypophyse, der Nebennierenrinde, der Schilddrüse oder den Inselzellen des Pankreas ist die feine Regulation intrazellulärer Ca^{2+}-Spiegel essenziell für die Steuerung der Hormonfreisetzung, Zelldifferenzierung und Zellproliferation.[6,73,87,92] Eine Dysregulation der TRPC-Kanalaktivität könnte demnach direkte Auswirkungen auf hormonelle Signalwege haben und zur Entstehung endokriner Störungen beitragen.

1.2 Endokrine Organe und deren funktionelle Bedeutung

Die drei endokrinen Organe und ihre Funktionen

Die Nebenschilddrüse, Schilddrüse und das Pankreas sind zentrale Organe des endokrinen Systems. Sie spielen eine wesentliche Rolle bei der Regulation von Stoffwechselprozessen, der Homöostase und der Signalübertragung im Körper. Ihre Funktion basiert in hohem Maße auf einer präzisen Ca^{2+}-Signalweiterleitung, was TRPC-Kanäle zu potenziell wichtigen Akteuren in diesen Organen macht. Im Folgenden sollen die anatomischen, histologischen und physiologischen Funktionen dieser einzelnen Organe kurz dargestellt werden.

Nebenschilddrüse

Die Nebenschilddrüsen oder Glandulae parathyroideae sind kleine, in der Regel vier, vorhandene Drüsen, die dorsolateral der Schilddrüse liegen. Anatomisch befinden sich die oberen Nebenschilddrüsen in der Nähe des Ringknorpels, während die unteren Nebenschilddrüsen in Höhe des dritten bis vierten Trachealrings lokalisiert sind. Diese Drüsen sind meist in das Gewebe der Schilddrüse eingebettet und durch eine dünne Kapsel von dieser abgegrenzt (s. Abbildung 4a).[71]

Histologisch bestehen die Nebenschilddrüsen aus zwei Hauptzelltypen: den Hauptzellen und den oxyphilen Zellen (s. Abbildung 4b). Die Hauptzellen sind für die Produktion des Parathormons (PTH) verantwortlich und erscheinen in histologischen Schnitten relativ hell und mit wenig Zytoplasma. Im Gegensatz dazu sind die oxyphilen Zellen größer und enthalten mehr Zytoplasma, wobei ihre genaue Funktion noch nicht vollständig geklärt ist.[16]

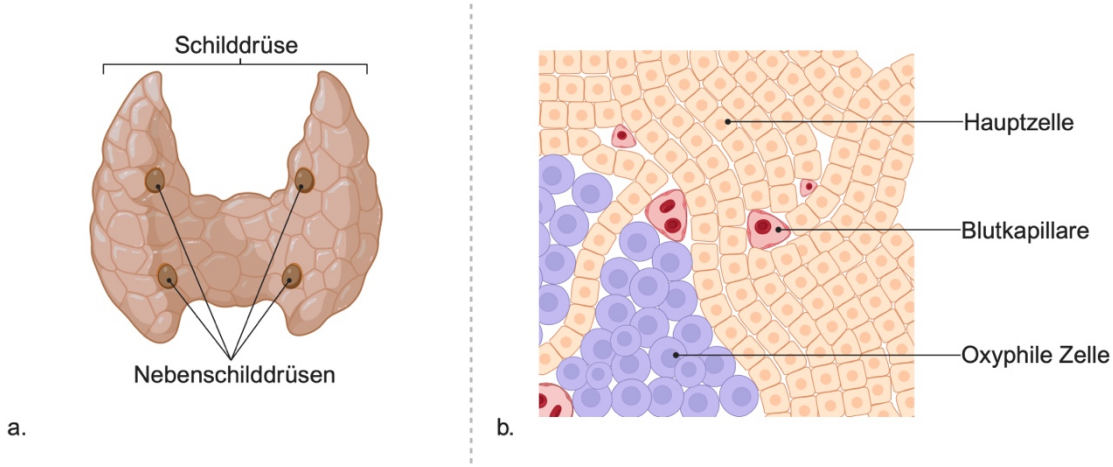


Abbildung 4: **Anatomie und Histologie der Nebenschilddrüse.**

a: Eine Darstellung der anatomischen Lage der Nebenschilddrüsen. Oberes und unteres Parathyreoideapaar an der dorsalen Schilddrüsenfläche.

b: Histologische Schemazeichnung der Nebenschilddrüse. Dicht aneinander gelagerte Hauptzellen umgeben von einem feinen Kapillarbett. Vereinzelt finden sich gruppiert Oxyphile Zellen.

Das Hormon PTH spielt eine zentrale Rolle in der Regulierung des Ca^{2+}- und Phosphathaushalts im Körper. Die Freisetzung von PTH wird primär durch den Ca^{2+}-Spiegel im Blut reguliert. Ein niedriger extrazellulärer Ca^{2+}-Spiegel reduziert die Aktivierung des calciumsensitiven Rezeptors (CaSR) auf den Hauptzellen der Nebenschilddrüse. Die daraus resultierende Abnahme des intrazellulären Ca^{2+}-Spiegels stimuliert die Exozytose von PTH.[15] Ein hoher extrazellulärer Ca^{2+}-Spiegel hingegen hemmt durch verstärkte CaSR-Aktivierung die Freisetzung von PTH über einen Anstieg des intrazellulären Ca^{2+}-Spiegels.[15] Damit unterscheiden sich die Hauptzellen der Nebenschilddrüse von anderen endokrine Zellen, in denen der Anstieg von Ca^{2+} typischerweise die Hormonfreisetzung fördert.[10] PTH wirkt auf verschiedene Organe, um den Ca^{2+}-Spiegel im Blut zu erhöhen. In den Knochen aktiviert PTH Osteoklasten, die den Knochenabbau anregen und dabei Ca^{2+} freisetzen. In den Nieren fördert PTH die Ca^{2+}-Resorption und steigert die Bildung von aktivem Vitamin D, welches wiederum die Ca^{2+}-Aufnahme im Darm erhöht.[57] Über diese Mechanismen trägt PTH dazu bei, den Ca^{2+}-Spiegel im Blut auf einem konstanten Niveau zu halten.[16,57] Zusätzlich zur direkten Wirkung auf Ca^{2+} reguliert Parathormon auch den Phosphatstoffwechsel, indem es die Phosphatausscheidung über die Nieren fördert.[57] Das Zusammenspiel von Parathormon, Calcitonin und Vitamin D3 ermöglicht eine feine Abstimmung des Ca^{2+}- und Phosphatgleichgewichts im Körper. Ein Ungleichgewicht in der Funktion der Nebenschilddrüsen, etwa durch eine Über- oder Unterproduktion von PTH, kann zu Erkrankungen wie Hyperparathyreoidismus oder Hypoparathyreoidismus führen. Diese Störungen wirken sich direkt auf den Ca^{2+}-Stoffwechsel aus und können zu schweren gesundheitlichen Problemen führen, wie etwa Knochenabbau, Nierensteinen oder Muskelkrämpfen.[57,70]

Schilddrüse

Die Schilddrüse ist eine endokrine Drüse, die im anterioren Halsbereich, direkt inferior des Kehlkopfs, lokalisiert ist. Sie besteht aus zwei symmetrischen Lappen, die durch einen schmalen Gewebestreifen, den Isthmus, miteinander verbunden sind (s. Abbildung 5a).[63] Die Schilddrüse ist von einer zweischichtigen Kapsel umgeben, wobei die innere Schicht mit dem Drüsengewebe verwachsen ist. Auf der dorsalen Seite der Schilddrüse befinden sich die vorher besprochenen Nebenschilddrüsen.[3]

Histologisch betrachtet besteht die Schilddrüse vor allem aus Follikeln, die von einer einschichtigen Epithelzellschicht, den Thyreozyten, umgeben sind (s. Abbildung 5b). Diese Follikel enthalten Kolloid, eine viskose Substanz, die die Vorstufen der Schilddrüsenhormone Tetraiodthyronin (T4) und Triiodthyronin (T3) speichert.[5] Zwischen den Follikeln finden sich die parafollikulären Zellen oder C-Zellen, die das Hormon Calcitonin produzieren (s. Abbildung 5b). Calcitonin spielt eine Rolle bei der Regulierung des Ca^{2+}-Spiegels im Blut, indem es die Ca^{2+}-Konzentration im Blut senkt, jedoch ist die genaue physiologische Bedeutung von Calcitonin beim Menschen nicht vollständig geklärt.[90]

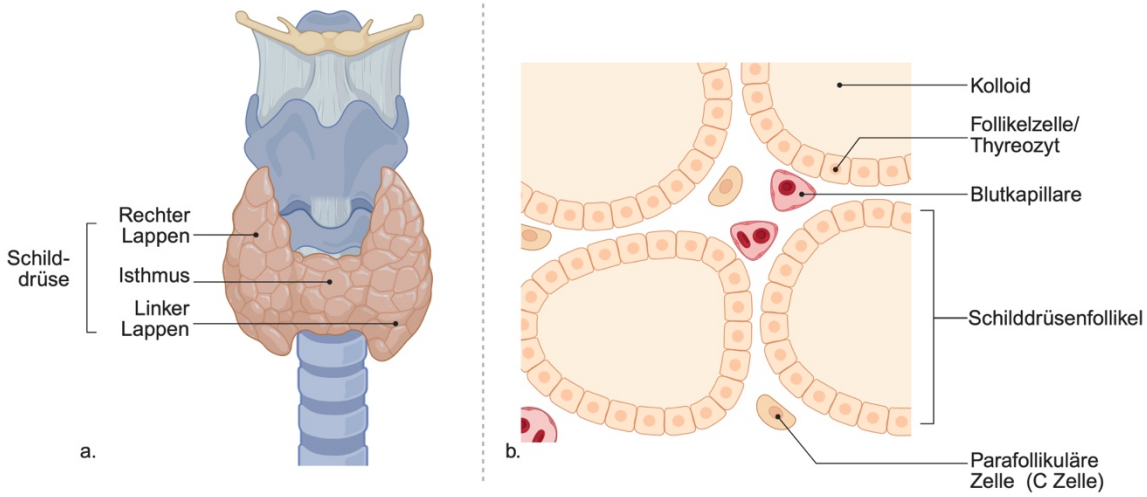


Abbildung 5: **Anatomie und Histologie der Schilddrüse.**
a: Eine Darstellung der anatomischen Lage der Schilddrüse mit beiden symmetrischen Schilddrüsenlappen.
b: Histologische Schemazeichnung der Schilddrüse. Eine einschichtige Follikelzellschicht, die Thyreozyten, die großflächig das Kolloid umgibt. Calcitonin-produzierende C-Zellen finden sich vereinzelt parafollikulär. Die Follikel sind umgeben von einem Kapillarbett.

Die Hauptfunktion der Schilddrüse besteht in der Produktion und Sekretion der Schilddrüsenhormone T3 und T4. T3 und T4 werden in den Follikeln synthetisiert.[13] Diese Hormone regulieren den Stoffwechsel, indem sie nahezu alle Körperzellen erreichen und deren Aktivität steigern.[5] T3 ist das biologisch aktivere Hormon, während T4 als Vorstufe fungiert und nach der Sekretion in T3 umgewandelt wird.[11] Die Synthese dieser Hormone wird durch das Thyreotropin (TSH) aus der Hypophyse reguliert, das seinerseits durch das Thyreotropin-Releasing-Hormon (TRH) des Hypothalamus gesteuert wird.[35] Ein Rückkopplungsmechanismus gewährleistet, dass der Spiegel von T3 und T4 im Blut konstant bleibt. Ein hoher Hormonspiegel hemmt die Freisetzung von TRH und TSH, während ein niedriger Hormonspiegel die Produktion von TSH anregt.[81]

Pankreas

Das Pankreas ist eine retroperitoneal gelegene Drüse, die sich quer über die hintere Bauchwand erstreckt und sich auf Höhe der Lendenwirbel 1 und 2 befindet.[62] Anatomisch gliedert sich das Pankreas in drei Hauptabschnitte: den Kopf, den Körper und den Schwanz. Der Pankreaskopf liegt eingebettet in der C-förmigen Krümmung des Duodenums, während der Schwanz in die Milz hineinragt. Der Ductus pancreaticus, der Hauptausführungsgang des Pankreas, verläuft vom Schwanz bis zum Kopf und mündet gemeinsam mit dem Gallengang in das Duodenum (s. Abbildung 6a).[24]

Histologisch setzt sich das Pankreas aus zwei funktionell unterschiedlichen Anteilen zusammen: dem exokrinen und dem endokrinen Anteil. Der exokrine Anteil bildet den Großteil des Pankreasgewebes und besteht aus Azinus-Zellen, die in kleinen Drüsenläppchen organisiert sind.[62] Diese Azini produzieren Verdauungsenzyme wie Amylase, Lipase und Proteasen.[103] Über ein verzweigtes Gangsystem werden diese Enzyme in den Ductus pancreaticus abgegeben und schließlich in das Duodenum transportiert.[43] Zwischen den Azini liegen die Langerhans-Inseln (s. Abbildung 6b).

Die Langerhans-Inseln beinhalten verschiedene hormonproduzierende Zelltypen, die zentrale Rollen unter anderem bei der Blutzuckerregulation übernehmen.[14] Die α-Zellen sezernieren Glukagon, ein Hormon, das die Freisetzung von Glukose aus der Leber stimuliert und so den Blutzuckerspiegel erhöht.[98] Die β-Zellen produzieren Insulin, das die Glukoseaufnahme in die Körperzellen fördert und den Blutzuckerspiegel senkt.[9] δ-Zellen geben Somatostatin ab, das sowohl die Insulin- als auch die Glukagonsekretion hemmt und so zur Regulation der Blutzuckerregulation beiträgt.[93] Die γ-Zellen bilden pankreatisches Polypeptid, das vermutlich an der Regulation der Verdauungsprozesse beteiligt ist, dessen genaue Rolle jedoch noch nicht vollständig geklärt ist.[89]

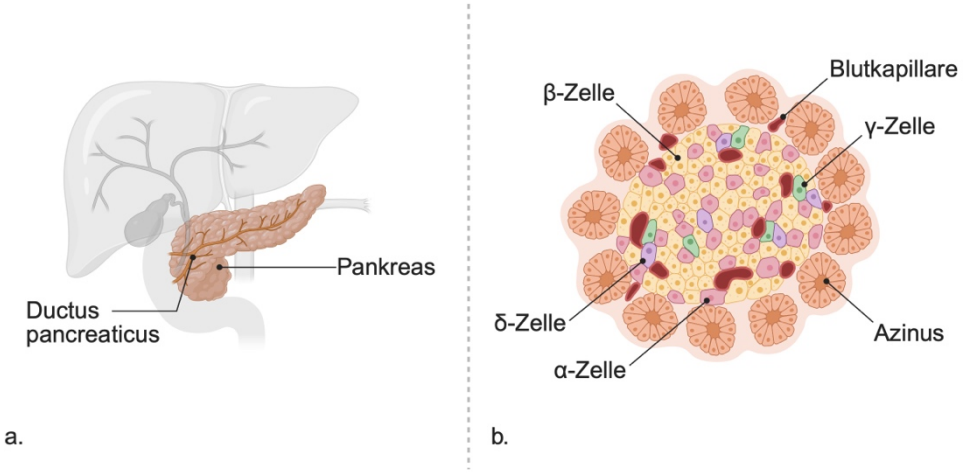


Abbildung 6: **Anatomie und Histologie des Pankreas.**

a: Eine anatomische Darstellung des Pankreas mit Ductus pancreaticus eingebettet in die C-förmige Krümmung des Duodenums.

b: Histologische Darstellung einer Langerhans-Insel umgeben von Azini mit den insulinproduzierenden β-zellen, den eher randständig gelegenen α-Zellen, sowie δ- und γ-Zellen, umgeben von einem Kapillarbett.

1.3 Aktueller Forschungsstand zu TRPC-Kanälen in endokrinen Organen

TRPC-Kanäle, insbesondere TRPC3 und TRPC6, scheinen eine zentrale Rolle bei der Regulierung von Ca^{2+}-Signalen zu spielen, die für die hormonelle Funktion der endokrinen Organe von entscheidender Bedeutung sind. In endokrinen Organen gewinnen TRPC-Kanäle zunehmend an Bedeutung als kritische Regulatoren der Hormonausschüttung und der zellulären Homöostase. Allerdings sind detaillierte Untersuchungen zu ihrer spezifischen Funktion und Verteilung in endokrinen Geweben, v.a. in der Schild- und Nebenschilddrüse, jedoch noch limitiert.

TRPC-Kanäle in der Nebenschilddrüse

Die Funktion von TRPC-Kanälen in der Nebenschilddrüse ist bislang nur unzureichend erforscht. Insbesondere zu TRPC3 und TRPC6 liegen nur wenige spezifische Daten vor. Dennoch deuten einige Studien darauf hin, dass bestimmte Vertreter der TRPC-Familie eine potenzielle physiologische Relevanz in der Regulation der PTH-Sekretion haben könnten.
TRPC1 scheint hierbei eine besondere Rolle einzunehmen. Es wird angenommen, dass TRPC1 als Teil eines Ca^{2+}-abhängigen Regelkreises agiert, der die PTH-Freisetzung moduliert. Onopiuk *et al.* beschreiben, dass bei erhöhtem extrazellulärem Ca^{2+}-Spiegel der Calcium-sensing receptor (CaSR) TRPC1-Kanäle aktiviert, was zu einem Anstieg des intrazellulären Ca^{2+} führt und paradoxerweise die PTH-Sekretion hemmt.[10,78] Umgekehrt würde ein Herunterregulieren von TRPC1 mit einer verminderten Sekretionshemmung verbunden sein, wie es im Fall des Krankheitsbilds des primären Hyperparathyreoidismus (pHPT) beschrieben wird.[78] Auch wenn TRPC3/6 in der vorliegenden Studie nicht untersucht wurden, liefern andere Arbeiten Hinweise auf dessen mögliche Relevanz: Ibeh *et al.* beschreiben, dass TRPC3 als Effektormolekül des CaSR dienen könnte. Die Beobachtung liegt nahe, dass TRPC3 ebenfalls in die Regulation des intrazellulären Ca^{2+} und damit direkt in die Kontrolle der PTH-Sekretion involviert sein könnte.[31,53]
Weitere Hinweise auf die Expression und Funktion von TRPC-Kanälen in der Nebenschilddrüse liefert eine Studie von Lu *et al.* In dieser wurden in normalen und adenomatösen Nebenschilddrüsengeweben Transkripte der messenger-Ribonukleinsäure (mRNA) von TRPC1, TRPC4, TRPC6, Orai1 und STIM1 nachgewiesen, nicht jedoch von TRPC3, TRPC5 und TRPC7.[64] Dazu unterstützen Daten aus dem Human Protein Atlas (HPA) die Expression von TRPC3- und TRPC6-RNA-Transkripten in der humanen Nebenschilddrüse.[48,51]

TRPC-Kanäle in der Schilddrüse

Die Datenlage zur Expression und physiologischen Bedeutung von TRPC3- und TRPC6-Kanälen in der Schilddrüse ist bislang ebenfalls begrenzt. Nur wenige Studien liefern Hinweise auf eine potenzielle Beteiligung dieser Kanäle an den biochemischen Prozessen des Schilddrüsengewebes.

Laut den Datensätzen des HPA lassen sich TRPC3- und TRPC6-RNA-Transkripte in Schilddrüsengewebe nachweisen[49,52]. Funktionelle Aussagen lassen sich aus diesen Daten jedoch nicht direkt ableiten. Eine experimentelle Studie von Asghar *et al.* liefert weiterführende Erkenntnisse: Die Autoren konnten mittels qualitativer End-Point-Polymerasekettenreaktion (PCR) sowohl TRPC3 als auch TRPC6 in gesundem menschlichem Schilddrüsengewebe nachweisen. Darüber hinaus identifizieren sie weitere TRPC-Kanäle, wie TRPC1, TRPC4 und TRPC5.[7] Ein besonderes Augenmerk galt wiederrum TRPC1, dessen Beteiligung am rezeptorgesteuerten Ca^{2+}-Einstrom in thyreoidalen Zellen verdeutlicht wurde.[7] Die Bedeutung dieser Kanäle konnte auch durch Zellinien-Versuche unterstrichen werden: In Schilddrüsenzelllinien der Ratte, die ausschließlich TRPC2 exprimieren, wurde eine vergleichbare Funktion für den TRPC-Kanal beobachtet.[94] Zudem konnten Asghar *et al.* zeigen, dass TRPC1 auch in der Pathophysiologie der Schilddrüse eine Rolle spielen könnte. In von Schilddrüsenkrebs entspringenden Zelllinien führte eine Herunterregulation von TRPC1 zu einer signifikanten Reduktion der Zellmigration und Proliferation, was auf eine Beteiligung von TRPC1 an tumorrelevanten Signalwegen hindeutet.[7]

TRPC-Kanäle im Pankreas

Im Vergleich zu den beiden vorherigen Kapiteln, sind die TRPC3- und TRPC6-Kanäle relativ gut im Pankreas erforscht und erfüllen entscheidende Rollen sowohl in seiner exokrinen als auch endokrinen Funktion.

Im exokrinen Anteil des Pankreas konnten TRPC3-Kanäle als ein essenzieller Baustein bei der Ca^{2+}-Regulation in Azinus-Zellen identifiziert werden: Kim *et al.* konnten zeigen, dass TRPC3 wesentlich an der Regulation des intrazellulären Ca^{2+}-Spiegels beteiligt ist und dadurch die Exozytose von Verdauungsenzymen steuert.[58,59] Die Relevanz von TRPC3 zeigte sich zudem auch in pathophysiologischen Modellen: Eine Deletion des Kanals führte zu einer Reduktion des krankhaften, anhaltenden Ansteigens des zytosolischen Ca^{2+}-Spiegels, verminderte die intrazelluläre Aktivierung von Trypsin und schwächte die Schwere einer akuten Pankreatitis ab.[59] Auch TRPC6 wurde in pankreatischen Azinus-Zellen nachgewiesen und übernimmt dort ähnliche Funktionen wie TRPC3. Kim *et al.* identifizierten TRPC6 in pankreatischen Azinus-Zellen von Mäusen und beschrieben seine Beteiligung an der Ca^{2+}-Regulation und Enzymfreisetzung.[58] In einer weiterführenden Studie identifizierten Du *et al.* eine regulatorische Rolle von Mikro-RNA-26a (miR-26a), die TRPC3 und TRPC6

herunterreguliert.[27] Im endokrinen Anteil des Pankreas ist die Rolle von TRPC3 weniger eindeutig. Einige Studien berichten, dass TRPC3 nicht in pankreatischen β-Zellen exprimiert wird.[67,86] Eine andere Studie hingegen zeigte eine TRPC3-abhänige Erhöhung des zytosolischen Ca^{2+}-Spiegels und eine damit verbundene Stimulation der Insulinsekretion in Ratten-β-Zellen.[104] Eine weitere Studie wies die Expression von TRPC3 sowohl in β- wie auch in α-Zellen nach.[83] TRPC6 hingegen wurde insbesondere zusammen mit TRPC3 mit der Proliferation von α- und β-Zellen in Verbindung gebracht, wobei eine direkte Beteiligung dieses Kanals an der Insulinsekretion bisher nicht eindeutig nachgewiesen werden konnte.[42] Nach den Datensätzen des HPA sowie konsolidierter Transkriptionsanalysen lassen sich TRPC6-RNA-Transkripte im Pankreasgewebe nachweisen[50], TRPC3-RNA-Transkripte [47] im Gegensatz zu oben erwähnten Studien jedoch nicht.

1.4 Zielsetzung der Dissertation und Forschungsfragen

Die Zielsetzung dieser Arbeit besteht in der systematischen Charakterisierung der Expression der TRPC-Kanäle TRPC3 und TRPC6 in den endokrinen Organen Nebenschilddrüse, Schilddrüse und Pankreas des Menschen. Im Fokus steht dabei die Detektion und Lokalisation der Proteinexpression der Kanäle innerhalb dieser Gewebe, um ein genaueres Verständnis für die potenzielle physiologische Bedeutung der Kanäle im Kontext hormoneller Regulation zu ermöglichen. Besonders relevant ist dabei die Untersuchung im humanen Gewebe, da viele bisherige Daten zu TRPC-Kanälen aus Tiermodellen stammen und die Übertragbarkeit auf den Menschen nicht selbstverständlich ist. Die Untersuchungen tragen dazu bei, bislang unzureichend erforschte Aspekte der TRPC-Kanalverteilung in hormonproduzierenden Organen zu beleuchten und einen Beitrag für weiterführende Studien zu ihrer Rolle im endokrinen System zu leisten.

Die vorliegende kumulative Promotionsarbeit orientiert sich an der folgenden übergeordneten Forschungsfrage:

> Werden die TRPC-Kanäle 3 und 6 in der menschlichen Nebenschilddrüse, Schilddrüse und Pankreas exprimiert?

Daraus ergeben sich die folgenden erkenntnisleitenden Unterfragen:

(1) Lassen sich spezifisch immunhistochemisch die Proteine TRPC3 und TRPC6 in diesen Organen nachweisen?
(2) Sind die Proteine TRPC3 und TRPC6 in bestimmten Zelltypen oder Regionen der Organe bevorzugt lokalisiert?
(3) Gibt es Unterschiede in der Expression von TRPC3 und TRPC6 innerhalb der untersuchten Organe?

Zusätzlich wurde in Publikation 1 das Expressionsmuster von TRPC3 und TRPC6 an pathologischem Gewebe untersucht, wodurch sich eine zusätzliche Forschungsfrage ergab:

(4) Unterscheiden sich die Expressionsmuster von TRPC3 und TRPC6 in gesunden Nebenschilddrüsen im Vergleich zu Nebenschilddrüsen von Patienten mit primärem Hyperparathyreoidismus?

2. Methodik der Studien

Alle drei Studien beruhen auf der gleichen grundlegenden Methode der Immunhistochemie, um die Expressionsmuster von TRPC3- und TRPC6-Proteinen zu untersuchen. Die detaillierten methodischen Vorgehensweisen und die verwendeten Materialien sind in den Publikationen 1-3 (s. Seiten 21-52) hinterlegt. Die verwendeten Gewebeproben stammen alle aus menschlichem Gewebe, entweder von Körperspendern des Anatomischen Instituts der Universität des Saarlandes oder von Patienten, denen Gewebeproben im Rahmen chirurgischer Eingriffe entnommen und am Institut für Allgemeine und Spezielle Pathologie des Universitätsklinikums des Saarlandes untersucht wurden. Bis auf gezielt zu Vergleichszwecken verwendete Proben aus pathologischem Gewebe wurden alle Gewebeproben als gesund und altersentsprechend eingestuft. Alle Untersuchungen wurden anonym durchgeführt, von der Ethikkommission der Ärztekammer des Saarlandes (163/20, 130/21) genehmigt und in Übereinstimmung mit den Richtlinien der Deklaration von Helsinki durchgeführt. Das Einverständnis der Patienten und der Körperspender wurde vorab eingeholt.

3. Überblick über die Publikationen

3.1 Publikation 1: “TRPC3 is Downregulated in Primary Hyperparathyroidism”

International Journal of
Molecular Sciences

MDPI

Article

TRPC3 Is Downregulated in Primary Hyperparathyroidism

Emilie Kirstein [1], Dirk Schaudien [2], Mathias Wagner [3], Coline M. Diebolt [1], Alessandro Bozzato [4], Thomas Tschernig [1,*] and Colya N. Englisch [1]

1 Institute for Anatomy and Cell Biology, Saarland University, 66421 Homburg, Germany; colya.englisch@uni-saarland.de (C.N.E.)
2 Fraunhofer Institute for Toxicology and Experimental Medicine, 30625 Hanover, Germany
3 Department of Pathology, Saarland University, 66421 Homburg, Germany
4 Department of Otorhinolaryngology, Head and Neck Surgery, Saarland University, 66421 Homburg, Germany; alessandro.bozzato@uks.eu
* Correspondence: thomas.tschernig@uks.eu

Abstract: Transient receptor potential canonical sub-family channel 3 (TRPC3) is considered to play a critical role in calcium homeostasis. However, there are no established findings in this respect with regard to TRPC6. Although the parathyroid gland is a crucial organ in calcium household regulation, little is known about the protein distribution of TRPC channels—especially TRPC3 and TRPC6—in this organ. Our aim was therefore to investigate the protein expression profile of TRPC3 and TRPC6 in healthy and diseased human parathyroid glands. Surgery samples from patients with healthy parathyroid glands and from patients suffering from primary hyperparathyroidism (pHPT) were investigated by immunohistochemistry using knockout-validated antibodies against TRPC3 and TRPC6. A software-based analysis similar to an H-score was performed. For the first time, to our knowledge, TRPC3 and TRPC6 protein expression is described here in the parathyroid glands. It is found in both chief and oxyphilic cells. Furthermore, the TRPC3 staining score in diseased tissue (pHPT) was statistically significantly lower than that in healthy tissue. In conclusion, TRPC3 and TRPC6 proteins are expressed in the human parathyroid gland. Furthermore, there is strong evidence indicating that TRPC3 plays a role in pHPT and subsequently in parathyroid hormone secretion regulation. These findings ultimately require further research in order to not only confirm our results but also to further investigate the relevance of these channels and, in particular, that of TRPC3 in the aforementioned physiological functions and pathophysiological conditions.

Keywords: TRPC3; TRPC6; CaSR; parathyroid gland; primary hyperparathyroidism; human; immunohistochemistry

Citation: Kirstein, E.; Schaudien, D.; Wagner, M.; Diebolt, C.M.; Bozzato, A.; Tschernig, T.; Englisch, C.N. TRPC3 Is Downregulated in Primary Hyperparathyroidism. *Int. J. Mol. Sci.* **2024**, *25*, 4392. https://doi.org/10.3390/ijms25084392

Academic Editor: Maurizio Delvecchio

Received: 2 March 2024
Revised: 8 April 2024
Accepted: 12 April 2024
Published: 16 April 2024

1. Introduction

The parathyroid gland is an endocrine organ that is allocated fourfold to the posterior surface of the thyroid gland [1]. Its main function is to synthetize and secrete parathyroid hormone, a task which is fulfilled by so-called chief cells [1]. Parathyroid hormone is an important effector in calcium homeostasis, which is basically responsible for increasing serum calcium levels when these are low [1]. Its secretion is dependent upon meticulous serum calcium sensing, ultimately forming a negative feedback loop [2,3]. In addition to the calcium-sensing receptor (CaSR), a homodimeric family C G protein-coupled receptor [4], there is evidence of further molecular players of critical interest in this context. For instance, transient receptor potential canonical subfamily channel 1 (TRPC1) has been demonstrated to be critical for parathyroid hormone release [2,5]. The hypothesis is that increased serum calcium levels activate the CaSR, directly, receptor-operated, or indirectly, store-operated, activating TRPC1 and thus enhancing inhibitory Ca^{2+} influx [2]. Parathyroid chief cells are indeed rather unique in view of the fact that, in most cell types, increased intracellular calcium levels do not inhibit but, on the contrary, actually trigger exocytosis [6]. However,

the exact pathway of regulation of parathyroid hormone release remains unknown [3]. In addition to chief cells, so-called oxyphilic cells also exist, although their exact function remains unclarified [7].

The second most frequent endocrinological disease after diabetes mellitus remains primary hyperparathyroidism (pHPT) [8]. Briefly, it is defined by increased serum calcium and parathyroid hormone levels with unknown cause, often found in combination with parathyroid adenomas [9]. Increased calcium levels may have a negative impact on several systems, including the kidneys and bones, and can therefore promote chronic kidney disease [10]. While mild pHPT might not manifest itself clinically for some time, severe pHPT can frequently lead to symptoms such as nausea, dehydration, muscle weakness, cognitive dysfunction, etc. [4].

Although the exact pathophysiology of pHPT is not clear [4,9], there is evidence that TRP channels from the canonical subfamily (TRPC1–7) might be involved [2,5]. They are tetramers, with each monomer being composed of six transmembrane segments and two cytoplasmic domains. The loop between the fifth and sixth transmembrane segments of each monomer contributes to ion pore formation, which is permeable to mono- and bivalent cations [11]. Being finely regulable, TRPC channels, including TRPC3 and TRPC6, are known to act in certain conditions as store- or receptor-operated channels (SOC or ROC) [12]. In the context of calcium homeostasis, TRPC3 has already been shown to play a critical role in renal calcium resorption. For instance, proximal tubular TRPC3 was demonstrated to be nephroprotective in experimental designs of hypercalciuria and nephrolithiasis, as reviewed by Englisch et al. [13]. In this context, we recently presented evidence of the tubular expression of TRPC3 in human specimens [14]. This calcium crosslink between both the kidney and the parathyroid gland [15], and the known potential of TRPC3 to mediate SOC- or ROC-based calcium entry, prompted our interest to investigate TRPC3 protein expression in human parathyroid glands. Indeed, a search in the online library "PubMed" with the keywords "parathyroid" and "TRPC" only provided less than ten results (March 2024), with none of these publications addressing TRPC3 or TRPC6 protein expression in the human parathyroid gland. As a matter of fact TRPC6 possesses abundant protein sequence analogies with TRPC3 [16,17] and displays wide disease implications [13,18]; thus, we aimed to investigate the presence of both TRPC3 and TRPC6 proteins in healthy and diseased human parathyroid tissues. In conclusion, the TRPC3 and TRPC6 proteins are expressed in both chief and oxyphilic cells of the healthy parathyroid gland. Our results further indicate a TRPC3 downregulation in pHPT, ultimately suggesting a critical role for TRPC3 in parathyroid gland physiology and pHPT pathophysiology.

2. Materials and Methods

2.1. Samples

Parathyroid gland tissue was obtained from surgery specimens, with informed consent provided by human adults who had either been operated due to pHPT ($n = 4$) or due to another cause not affecting the parathyroid gland ($n = 4$). Patient demographics are presented in Table 1. Samples from the pHPT group displayed histologically secured hyperplasia or adenoma and clinically indicated pHPT. The healthy samples displayed no pathologies and were age appropriate as evaluated and labeled by trained pathologists. Three of the samples of healthy parathyroid tissue were obtained from female patients and one from a male patient. The mean age was 48.3 with an SD of 18.9 years at the surgery time point. Samples with pHPT were obtained from four female patients with no known genetic phenotypes of pHPT. The mean age was 52.8 with an SD of 6.3 years at the surgery time point. The research was conducted anonymously, approved by the Ethics Committee of the Saarland Medical Association (130/21), and performed in accordance with the guidelines of the Declaration of Helsinki.

Table 1. Overview of patient demographics. Tissue type (healthy or from primary hyperparathyroidism [pHPT]), age, sex, and surgery indication/pathology are listed for each tissue sample.

Tissue	Age	Sex	Surgery Indication/Pathology
Healthy	23	Female	Hashimoto Thyroiditis (Lymphofollicular Hyperplasia)
Healthy	68	Female	Struma Nodosa (Follicular Lymphocytic Thyroiditis)
Healthy	37	Female	Unspecified Thyroid Pathology
Healthy	65	Male	Struma Multinodosa
pHPT	48	Female	Nodular Hyperplasia of Parathyroid Gland
pHPT	47	Female	Mild diffuse Hyperplasia of Parathyroid Gland
pHPT	63	Female	Parathyroid Adenoma
pHPT	53	Female	Nodular Hyperplasia of Parathyroid Gland

2.2. Tissue Treatment

After surgical removal, the parathyroid gland samples were immediately fixed in 4% formalin, where they were kept for 24 h at 4 °C. They were then switched to phosphate-buffered saline for 24 h at 4 °C, exposed to flowing water for 3 h, and incubated in 70% isopropanol for the same period (Otto Fischar GmbH & Co., Saarbrücken, Germany). Afterwards, the following incubation steps were conducted using a mechanical tissue embedder (SLEE medical GmbH, Mainz, Germany): 70% isopropanol (3 h), 80% isopropanol (90 min), 90% isopropanol (90 min), 100% isopropanol (2 × 90 min), methyl benzoate (3 × 90 min; Thermo Fisher Scientific Inc., Waltham, MA, USA), and liquid paraffine (2 × 2 h; Carl Roth GmbH & Co., KG, Karlsruhe, Germany). Later, the samples were sectioned at a thickness of 4 µm and mounted on glass slides.

2.3. Histology

Standard hematoxylin and eosin-stained sections were prepared (H&E). Briefly, samples were serially rehydrated through incubation in 100% xylol (15 min; VWR International, Fontenay-sous-Bois, France), followed by decreasing concentrations of isopropanol solutions (100% [10 min], 90% [5 min], 80% [5 min]; Central Chemical Storage, Saarland University, Saarbrucken, Germany), and stained with Ehrlich's hematoxylin (8 min; Carl Roth GmbH & Co., KG, Karlsruhe, Germany). After washing in distillated water and bluing in fluent water for 12 min, staining with 0.1% eosin (210 s; Central Chemical Storage, Saarland University, Saarbrucken, Germany) was performed and adjusted in 90% isopropanol. Finally, the samples were dehydrated using 100% isopropanol (10 min) and 100% xylol (15 min).

For immunohistochemical staining of TRPC3, TRPC6, and CaSR, the paraffin was removed, and antigen recovery was performed through citrate buffer incubation for 60 min at 95 °C. The samples were incubated with the primary antibody overnight and at room temperature (polyclonal rabbit anti-TRPC3, lyophilized, ACC-016, 1:50, Alomone Labs, Jerusalem BioPark, Jerusalem Israel; polyclonal rabbit anti-TRPC6, lyophilized, ACC-017, 1:50, Alomone Labs, Jerusalem BioPark, Israel; polyclonal rabbit anti-CaSR, lyophilized, ACR-004, 1:100, Alomone Labs, Jerusalem BioPark, Israel). Instead of the primary antibody, 1:500 diluted rabbit serum was used for the negative control. A peroxidase-labeled secondary antibody (HRP, horseradish peroxidase, anti-rabbit goat, A10547; Invitrogen AG, Carlsbad, CA, USA) and diaminobenzidine tetrahydrochloride as chromogen (DAB; incubation time = 3 min for TRPC3 and 6, incubation time = 4 min for CaSR; SK-4103 Vector Laboratories, Burlingame CA, USA) were added to trace the primary antibody. Nuclear counterstaining with hematoxylin followed (C. Roth, Karlsruhe, Germany). The anti-TRPC3, anti-TRPC6, and anti-CaSR antibodies were designed to detect the corresponding channels in mouse, rat, and human tissues, as annotated by the manufacturer (Alomone Labs, Jerusalem BioPark, Israel). Moreover, the anti-TRPC3 antibody (Peptide HKLSEKLNPSVLRC) was designed to detect the amino acid residues 822–835 at the intracellular COOH-terminus of mouse TRPC3, whereas the anti-TRPC6 antibody (Peptide [C]RRNESQDYLLMDELG) detected the amino acid residues 24–38 of the intracelluar

N-terminus of mouse TRPC6 (Alomone Labs, Jerusalem BioPark, Jerusalem Israel). The anti-CaSR antibody (Peptide [C]DDYGRPGIEKFREE) recognized the amino acid residues 216–229 at the extracellular N-terminus of human CaSR. The molecular structures of TRPC3 and TRPC6 as well as CaSR are described elsewhere [19,20]. The anti-TRPC antibodies were knockout-validated, as indicated by the manufacturer [21–25].

2.4. Evaluation

The Nano Zoomer S210 (Hamamatsu, Japan) was used to digitize the slides. Microphotographs were captured using NDP.view2 image viewing software from Hamamatsu (U12388-01, Hamamatsu, Japan). A semiquantitative analysis of the DAB staining was conducted using Visiopharm image analysis software (version 01.2023, Visiopharm, Denmark). Each slide had a manually annotated region of interest (ROI) with no marginal artefacts. To ensure accurate recognition of histological structures, literature descriptions such as in [1] were consulted and control examinations conducted by trained anatomists.

A threshold system was established to classify the DAB-positive areas within the ROI, whereby the detection of these DAB-positive areas was facilitated by the DAB filter incorporated in Visiopharm software. The threshold value was divided into three sections: between the darkest brown staining (20) and the lightest visible staining (160). Areas with the darkest DAB stain (values between 20–80) were designated as +3, mid-range values (between 81–120) as +2, and the lightest DAB-stained areas (between 121–160) as +1. Furthermore, connective, or fatty tissue was excluded by assigning an extra section (between 0 and 200) with a value of 0–1. These areas, along with hematoxylin staining, were considered DAB-negative. Weighted DAB-positive areas per slide were calculated by multiplying the DAB-positive areas by their respective attributed value (1, 2, or 3) and summing up all values. This value was normalized to the measured tissue area (sum of DAB-negative and DAB-positive areas), resulting in a normalized weighted DAB area score between 0 and 3, akin to a pixelwise H-score (Figure 1) [26]. The positive proportion presented the sum of all DAB-positive areas in relation to the measured tissue area (sum of DAB-positive areas and DAB-negative areas).

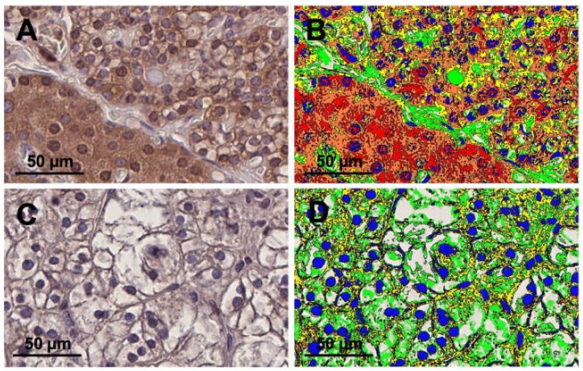


Figure 1. Immunohistochemical staining with an anti-TRPC3 antibody of the human parathyroid gland with and without image analysis. The upper two panels (**A**,**B**) illustrate microphotographs from healthy human parathyroid tissue. The lower two panels (**C**,**D**) present microphotographs from human parathyroid tissue from a patient with primary hyperparathyroidism (pHPT). Panels (**A**,**C**) are without image analysis and panels (**B**,**D**) are with image analysis. Panels (**B**,**D**) show semiquantitative diaminobenzidine tetrahydrochloride (DAB) color scoring. The color red represents a DAB score of 3, orange a DAB score of 2, and yellow a DAB score of 1. The color green represents subtracted connective and fatty tissues. The color blue shows the subtracted hematoxylin area. Both were attributed as DAB-negative. Panels (**A**–**D**) have 40× software magnification and a scale bar of 50 µm.

2.5. Statistical Analysis

Statistical analysis was performed using GraphPad Prism software (version 10.1.0). Descriptive statistics were provided using the mean value and the standard deviation (mean ± SD). The Mann–Whitney *U* test was used for comparisons between two independent cohorts. *p*-values (*p*) are two-sided and were considered significant when <0.05.

3. Results

Representative H&E staining of healthy parathyroid tissue as well as tissue from pHPT patients is shown in Figure 2. It displays the morphology of chief and oxyphilic cells in both healthy and pHPT tissues. In the description below, the positive proportion (%) is given first followed by the normalized weighted DAB area score, as detailed in Section 2.4.

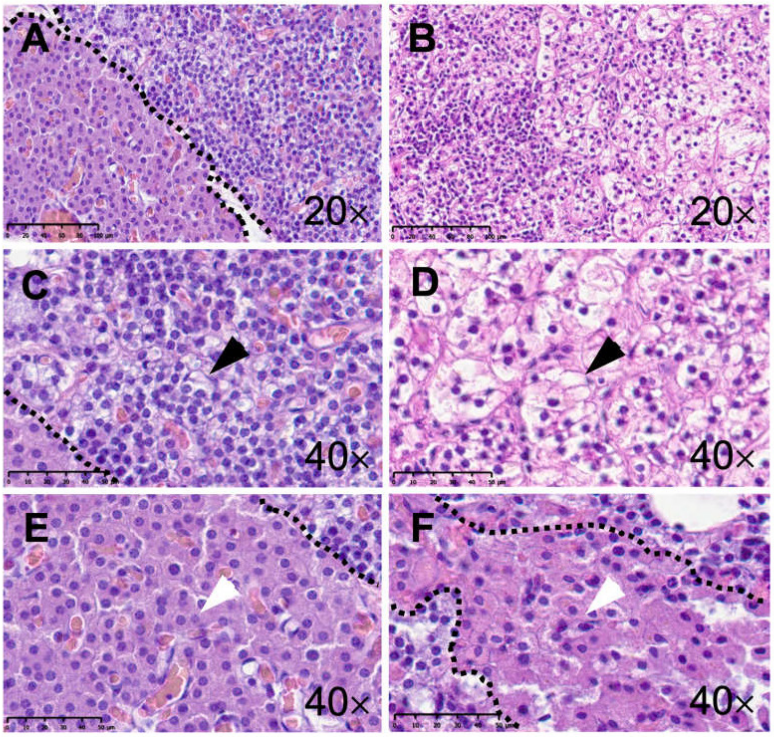


Figure 2. Hematoxylin and eosin staining of human parathyroid tissue. The left column (A,C,E) displays microphotographs from healthy parathyroid tissue and the right column (B,D,F) shows tissue from patients with primary hyperparathyroidism (pHPT). Panel (A) features an overview of healthy parathyroid tissue with a pool of chief cells on the right side and a pool of oxyphilic cells on the left side of the demarcation (scale bar: 100 µm). Panels (C,E) display respectively chief (black arrow) and oxyphilic (white arrow) cells at higher magnification (scale bar: 50 µm). Panel (B) displays an overview of human parathyroid tissue from a patient with pHPT (scale bar: 100 µm). Panels (D,F) display respectively chief (black arrow) and oxyphilic (white arrow) cells at higher magnification (scale bar: 50 µm).

Human parathyroid gland tissue displayed immunostaining in all samples after incubation with the anti-TRPC3, anti-TRPC6, and anti-CaSR antibodies. However, obvious contrasts can be described. Healthy parathyroid gland tissue featured anti-TRPC3 staining

in both chief and oxyphilic cells in all samples (68 ± 5%; 1.12 ± 0.17; Figure 3A,C). Oxyphilic cells, in particular, revealed pronounced immunoreactivity (Figure 3C). By contrast, all of the samples from pHPT patients presented much weaker immunoreactivity to the anti-TRPC3 antibody (37 ± 7%, $p = 0.03$; 0.44 ± 0.11, $p = 0.03$; Figure 3B,D). Interestingly, one pHPT sample exhibited a somewhat stronger staining pattern than the other three. Only in this sample, oxyphilic cells were detectable, although immunoreactivity to the anti-TRPC3 antibody was still weaker than in healthy parathyroid tissue. Negative controls upon TRPC3 staining displayed no immunoreactivity in both one healthy (4%; 0.04; Figure 3E and one diseased sample (2%; 0.02; Figure 3F).

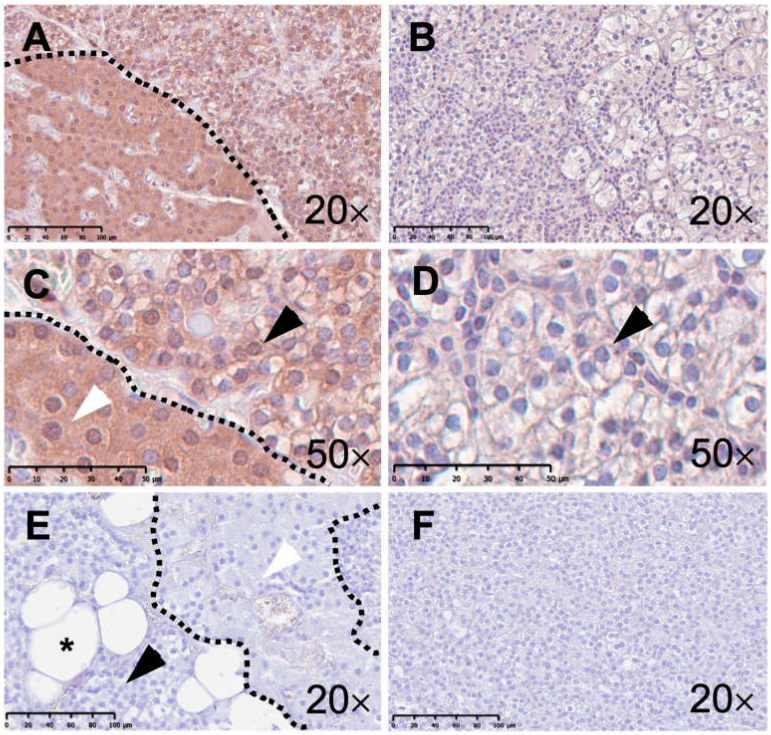


Figure 3. Immunohistochemical staining with an anti-TRPC3 antibody of the human parathyroid gland. The left column (A,C,E) illustrates microphotographs from healthy human parathyroid tissue. The right column (B,D,F) presents microphotographs from human parathyroid tissue from patients with primary hyperparathyroidism (pHPT). Panel (A) displays an overview of healthy parathyroid tissue with a pool of chief cells on the right side and a pool of oxyphilic cells on the left side of the demarcation (scale bar: 100 µm). Panel (C) displays both chief (black arrow) and oxyphilic (white arrow) cells at higher magnification (scale bar: 50 µm). Panel (B) displays an overview of human parathyroid tissue from a patient with pHPT (scale bar: 100 µm). Panel (D) displays chief cells (black arrow) at higher magnification (scale bar: 50 µm). Panel (E) displays an overview of the negative control staining of healthy parathyroid tissue. Chief (black arrow), oxyphilic (white arrow), and fat cells (asterisk) are displayed (scale bar: 100 µm). Panel (F) displays an overview of the negative control staining of parathyroid tissue from a patient with pHPT (scale bar: 100 µm).

On the other hand, anti-TRPC6 staining was similar in healthy (73 ± 3%; 1.59 ± 0.23; Figure 4A,C) and diseased tissues from pHPT patients (68 ± 6%, $p = 0.2$; 1.36 ± 0.30, $p = 0.3$;

Figure 4B,D). Negative controls upon TRPC6 staining displayed no immunoreactivity in both one healthy (3%; 0.03; Figure 4E) and one diseased sample (2%; 0.02; Figure 4F).

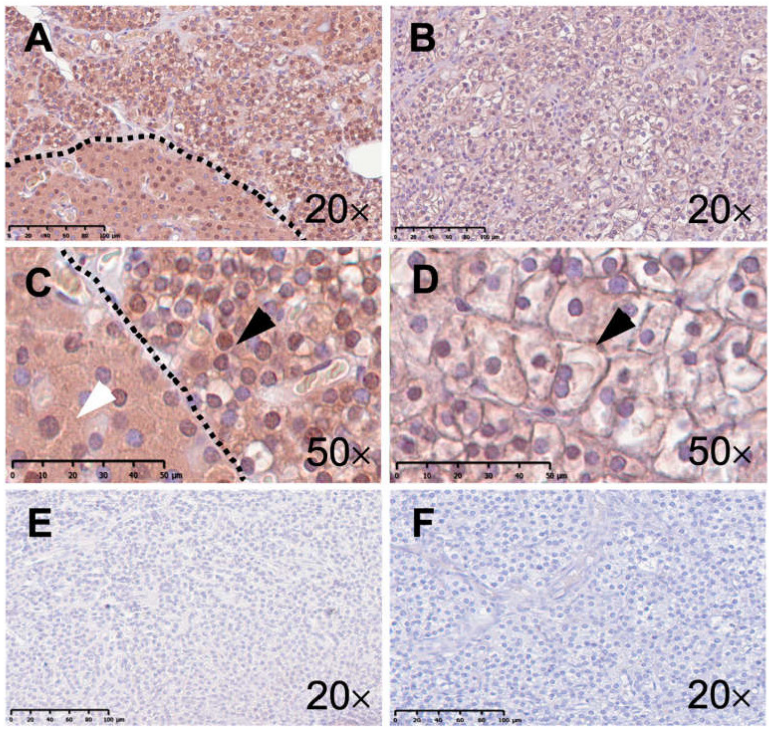


Figure 4. Immunohistochemical staining with an anti-TRPC6 antibody of the human parathyroid gland. The left column (A,C,E) illustrates microphotographs from healthy human parathyroid tissue. The right column (B,D,F) presents microphotographs from human parathyroid tissue from patients with primary hyperparathyroidism (pHPT). Panel (A) displays an overview of healthy parathyroid tissue with a pool of chief cells on the right upper side and a pool of oxyphilic cells on the left lower side of the demarcation (scale bar: 100 µm). Panel (C) displays both chief (black arrow) and oxyphilic (white arrow) cells at higher magnification (scale bar: 50 µm). Panel (B) displays an overview of human parathyroid tissue from a patient with pHPT (scale bar: 100 µm). Panel (D) displays chief cells (black arrow) at higher magnification (scale bar: 50 µm). Panel (E) displays an overview of negative control staining of healthy parathyroid tissue (scale bar: 100 µm). Panel (F) displays an overview of negative control staining of parathyroid tissue from a patient with pHPT (scale bar: 100 µm).

Additionally, CaSR immunoreactivity was featured in both healthy (Figure 5A,C) and diseased tissues from pHPT patients (Figure 5B,D). Healthy parathyroid tissue showed positive, yet inhomogeneous, immunoreactivity to the anti-CaSR antibody in all samples ($45 \pm 14\%$; 0.65 ± 0.32). In contrast, tissue from patients with pHPT demonstrated weaker, yet inhomogeneous, immunoreactivity to the anti-CaSR antibody compared to healthy analogous tissue ($21 \pm 12\%$, $p = 0.03$; 0.23 ± 0.13, $p = 0.03$). Negative controls displayed no immunoreactivity in both one healthy (6%; 0.07; Figure 5E) and one diseased sample (3%; 0.03; Figure 5F).

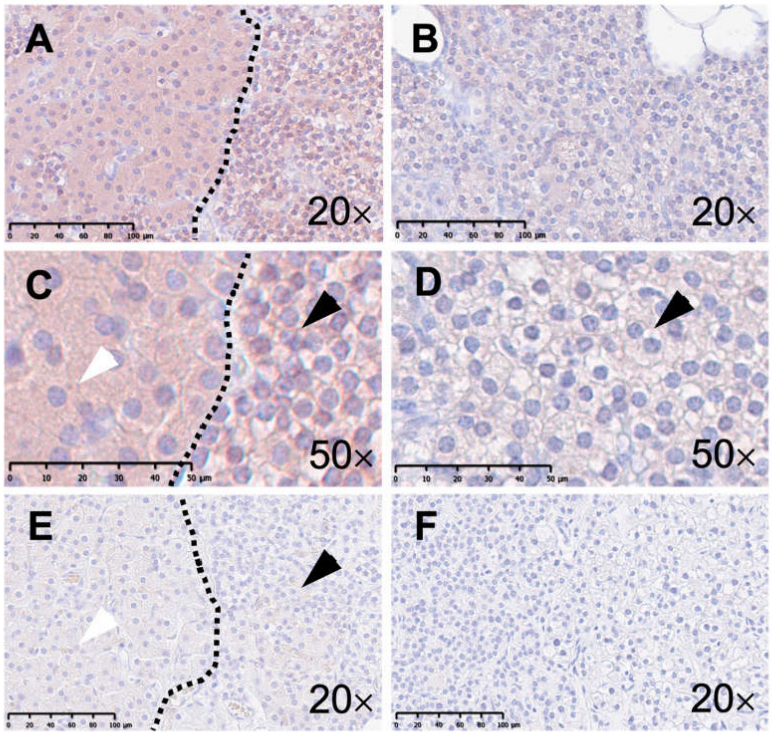


Figure 5. Immunohistochemical staining with an anti-CaSR antibody of the human parathyroid gland. The left column (A,C,E) illustrates microphotographs from healthy human parathyroid tissue. The right column (B,D,F) presents microphotographs from human parathyroid tissue from patients with primary hyperparathyroidism (pHPT). Panel (A) displays an overview of healthy parathyroid tissue with a pool of chief cells on the right side and a pool of oxyphilic cells on the left side of the demarcation (scale bar: 100 µm). Panel (C) displays both chief (black arrow) and oxyphilic (white arrow) cells at higher magnification (scale bar: 50 µm). Panel (B) displays an overview of human parathyroid tissue from a patient with pHPT (scale bar: 100 µm). Panel (D) displays chief cells (black arrow) at higher magnification (scale bar: 50 µm). Panel (E) displays an overview of the negative control staining of healthy parathyroid tissue. Chief (black arrow) and oxyphilic (white arrow) cells are displayed (scale bar: 100 µm). Panel (F) displays an overview of the negative control staining of parathyroid tissue from a patient with pHPT (scale bar: 100 µm).

4. Discussion

To the best of our knowledge, these findings present—for the first time—evidence indicating wide TRPC3 and TRPC6 protein expression in the human parathyroid gland. This includes both main and oxyphilic cells. We further observed that anti-TRPC3 and anti-CaSR—however not anti-TRPC6—immunolabeling were notably decreased in pHPT-diseased tissue. These findings ultimately suggest that TRPC3 plays a critical role in parathyroid hormone and calcium homeostasis next to the CaSR.

The sample-associated study limitations include the small group size and the respective age and sex variations, factors which possibly restrict the generalizability of our conclusions. Also, protein expression in healthy parathyroid tissue might have been affected by adjacent benign diseased thyroid tissue, although the tissue had been labeled as healthy and age appropriate by trained pathologists.

Immunohistochemistry is a highly specific method that not only enables protein identification but also a detailed histological description of its distribution [27]. However, western blot analysis, which can be restricted in use due to high premises toward the sample's nature and quality [28,29], could serve to further substantiate the precision of our data. Nevertheless, although immunohistochemistry cannot be quantified and is primarily a qualifying method, tools do exist to perform a semiquantitative analysis [26]. Moreover, such semiquantitative staining analyses are often correlated with western blot data, as observed previously [30,31].

One of the limitations of such a semiquantitative analysis, which could nevertheless affect the evaluation, is variation in sample size. However, there were only minor differences that did not relevantly distort the overall picture. The semiquantitative analysis is intended to be supportive, as, for example, in the case of the H-score, which presents a staining pattern which may correlate with antigen presence but cannot be equated with it [26]. Also, it does not provide any indication of the activity and integrity of the detected protein.

Interestingly, oxyphilic cells exhibited stronger staining than chief cells, possibly contributing to weaker global staining of diseased tissue given that oxyphilic cells were absent in three of the four pHPT samples. However, chief cells still showed weaker immunolabeling in diseased tissue compared to healthy tissue, making the difference clearly not only attributable to the mismatch in oxyphilic cell presence between the two groups.

In the study presented by Lu et al., reverse transcription polymerase chain reaction (RT-PCR) indicated that *trpc3* complementary deoxyribonucleic acid (cDNA)/messenger ribonucleic acid (mRNA) is not expressed in human parathyroid cells [5]. However, according to the HPA and consensus datasets from the Human Protein Atlas, TRPC3 RNA transcripts can be detected in the parathyroid gland [32,33]. Instead, Lu et al. detected an increase in TRPC1, 4, and 6 mRNA expression in adenomatous compared to healthy parathyroid tissue [5]. A critical limitation of these considerations was a relevant disbalance in cohort size [5]. In this context, it is insightful to observe that, in our study, anti-TRPC6-protein immunolabeling was similar in pHPT tissue in comparison to healthy parathyroid tissue. In contrast, anti-TRPC3 protein immunolabeling was significantly weaker in pHPT tissue compared to healthy tissue. In three of the four pHPT samples, staining seemed almost negative to the human eye, while in the fourth, it was slightly recognizable. Interestingly, anti-TRPC6 immunolabeling was also increased in this sample. This might be attributable to different factors, including artefacts. Following the hypothesis that increased immunostaining is correlated with increased antigen presence, our results would indicate a significant downregulation of TRPC3 protein expression in pHPT. Similar conclusions from immunohistochemistry have been drawn before, but with CaSR [34–36]. To our knowledge this has not been described before, all the more in view of the fact that, as previously mentioned, TRPC3 protein expression in the parathyroid gland was unclear. Considering that TRPC3 can act both as SOC and ROC, combined with its relevance in calcium homeostasis, as suggested in the kidney [13,14], a potential role in parathyroid hormone secretion or rather secretion regulation becomes conceivable. The study by Onopiuk et al. did not address TRPC3 but demonstrated that TRPC1 is such a molecular player downstream of CaSR that contributes to the suppression of parathyroid hormone secretion in response to increased serum calcium levels [2]. Inversely, TRPC1 downregulation would be associated with decreased secretion inhibition, as suggested in pHPT. A similar picture emerges from our investigations of human tissue with respect to TRPC3 protein. Moreover, TRPC3 is known to act as a downstream effector of CaSR, as summarized by Englisch et al. [13]. This is further supported by our results that also indicated CaSR downregulation in tissue from pHPT patients. This ultimately suggests an association between the downregulation of TRPC3 and of CaSR, which further underlines the potential relevance of TRPC3, not only in parathyroid physiology but also in pHPT pathophysiology. However, it remains unclear as to whether TRPC3 downregulation is involved in the emergence of pHPT or is only a manifestation of it. It thus becomes even more obvious that, in order to understand the molecular disease dynamics, the impact of

serum calcium and parathyroid hormone levels on protein expression, and the function of TRPC3 in the parathyroid gland, further experimental studies are required, which can, for example, include the use of calcimimetics [37].

Overall, further studies with higher case numbers are needed to confirm our results. In addition to the abovementioned experimental investigations, prospective clinical studies with a broad assessment covering a variety of biochemical and clinical data could shed light on the clinical relevance of TRPC3 expression regulation, as already carried out on CaSR [35]. Looking ahead, further differentiating studies are needed on genetic phenotypes and pathological presentations, such as, for example, parathyroid hyperplasia, adenoma, or carcinoma [36].

5. Conclusions

The studies by Lu et al. and Onopiuk et al. suggest that there is a clear need for research on the TRPC channels in the parathyroid gland [2,5]. The results we have presented here provide strong evidence for TRPC3 and TRPC6 protein expression in human parathyroid tissue. We describe weaker protein expression of TRPC3—together with CaSR—in tissue from pHPT samples, which suggests a critical function of TRPC3 in pHPT. Further studies are necessary to confirm our findings and to address them both experimentally and in clinical follow-up studies. Ultimately, TRPC3 might become a new target in the prevention or treatment of pHPT.

Author Contributions: Conceptualization, T.T. and C.N.E.; Data curation, C.N.E.; Formal analysis, C.N.E.; Investigation, E.K.; Methodology, E.K., D.S., C.M.D., T.T., and C.N.E.; Resources, M.W., A.B., and T.T.; Writing—original draft, E.K. and C.N.E.; Writing—review & editing, D.S., M.W., C.M.D., A.B., and T.T. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Saarland Medical Association (130/21) on 21 May 2021.

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Data can be obtained from the corresponding author upon reasonable request.

Conflicts of Interest: The authors declare no conflicts of interest.

References

1. Brown, M.B.; Limaiem, F. Histology, Parathyroid Gland. In *StatPearls*; StatPearls: Treasure Island, FL, USA, 2023.
2. Onopiuk, M.; Eby, B.; Nesin, V.; Ngo, P.; Lerner, M.; Gorvin, C.M.; Stokes, V.J.; Thakker, R.V.; Brandi, M.L.; Chang, W.; et al. Control of PTH secretion by the TRPC1 ion channel. *JCI Insight* **2020**, *5*, e132496. [CrossRef] [PubMed]
3. Brown, E.M. Role of the calcium-sensing receptor in extracellular calcium homeostasis. *Best Pract. Res. Clin. Endocrinol. Metab.* **2013**, *27*, 333–343. [CrossRef] [PubMed]
4. Minisola, S.; Arnold, A.; Belaya, Z.; Brandi, M.L.; Clarke, B.L.; Hannan, F.M.; Hofbauer, L.C.; Insogna, K.L.; Lacroix, A.; Liberman, U.; et al. Epidemiology, Pathophysiology, and Genetics of Primary Hyperparathyroidism. *J. Bone Miner. Res.* **2022**, *37*, 2315–2329. [CrossRef] [PubMed]
5. Lu, M.; Branstrom, R.; Berglund, E.; Hoog, A.; Bjorklund, P.; Westin, G.; Larsson, C.; Farnebo, L.O.; Forsberg, L. Expression and association of TRPC subtypes with Orai1 and STIM1 in human parathyroid. *J. Mol. Endocrinol.* **2010**, *44*, 285–294. [CrossRef] [PubMed]
6. Barclay, J.W.; Morgan, A.; Burgoyne, R.D. Calcium-dependent regulation of exocytosis. *Cell Calcium* **2005**, *38*, 343–353. [CrossRef] [PubMed]
7. Tanaka, Y.; Funahashi, H.; Imai, T.; Seo, H.; Tominaga, Y.; Takagi, H. Oxyphil cell function in secondary parathyroid hyperplasia. *Nephron* **1996**, *73*, 580–586. [PubMed]
8. Nilsson, I.L.; Yin, L.; Lundgren, E.; Rastad, J.; Ekbom, A. Clinical presentation of primary hyperparathyroidism in Europe–nationwide cohort analysis on mortality from nonmalignant causes. *J. Bone Miner. Res.* **2002**, *17* (Suppl. 2), N68–N74. [PubMed]
9. Walker, M.D.; Silverberg, S.J. Primary hyperparathyroidism. *Nat. Rev. Endocrinol.* **2018**, *14*, 115–125. [CrossRef] [PubMed]

10. Jha, S.; Simonds, W.F. Molecular and Clinical Spectrum of Primary Hyperparathyroidism. *Endocr. Rev.* **2023**, *44*, 779–818. [CrossRef] [PubMed]
11. Kaneko, Y.; Szallasi, A. Transient receptor potential (TRP) channels: A clinical perspective. *Br. J. Pharmacol.* **2014**, *171*, 2474–2507. [CrossRef] [PubMed]
12. Abramowitz, J.; Birnbaumer, L. Physiology and pathophysiology of canonical transient receptor potential channels. *FASEB J.* **2009**, *23*, 297–328. [CrossRef] [PubMed]
13. Englisch, C.N.; Paulsen, F.; Tschernig, T. TRPC Channels in the Physiology and Pathophysiology of the Renal Tubular System: What Do We Know? *Int. J. Mol. Sci.* **2022**, *24*, 181. [CrossRef] [PubMed]
14. Diebolt, C.M.; Schaudien, D.; Junker, K.; Krasteva-Christ, G.; Tschernig, T.; Englisch, C.N. New insights in the renal distribution profile of TRPC3—Of mice and men. *Ann. Anat.* **2023**, *252*, 152192. [CrossRef] [PubMed]
15. Moe, S.M. Calcium Homeostasis in Health and in Kidney Disease. *Compr. Physiol.* **2016**, *6*, 1781–1800. [PubMed]
16. Kiselyov, K.; Patterson, R.L. The integrative function of TRPC channels. *Front. Biosci. (Landmark Ed.)* **2009**, *14*, 45–58. [CrossRef] [PubMed]
17. Wang, H.; Cheng, X.; Tian, J.; Xiao, Y.; Tian, T.; Xu, F.; Hong, X.; Zhu, M.X. TRPC channels: Structure, function, regulation and recent advances in small molecular probes. *Pharmacol. Ther.* **2020**, *209*, 107497. [CrossRef] [PubMed]
18. Eder, P.; Groschner, K. TRPC3/6/7: Topical aspects of biophysics and pathophysiology. *Channels* **2008**, *2*, 94–99. [CrossRef] [PubMed]
19. Tang, Q.; Guo, W.; Zheng, L.; Wu, J.X.; Liu, M.; Zhou, X.; Zhang, X.; Chen, L. Structure of the receptor-activated human TRPC6 and TRPC3 ion channels. *Cell Res.* **2018**, *28*, 746–755. [CrossRef]
20. Hendy, G.N.; Canaff, L.; Cole, D.E. The CASR gene: Alternative splicing and transcriptional control, and calcium-sensing receptor (CaSR) protein: Structure and ligand binding sites. *Best Pract. Res. Clin. Endocrinol. Metab.* **2013**, *27*, 285–301. [CrossRef] [PubMed]
21. Xia, W.; Wang, Q.; Lin, S.; Wang, Y.; Zhang, J.; Wang, H.; Yang, X.; Hu, Y.; Liang, H.; Lu, Y.; et al. A high-salt diet promotes hypertrophic scarring through TRPC3-mediated mitochondrial $Ca^{(2+)}$ homeostasis dysfunction. *Heliyon* **2023**, *9*, e18629. [CrossRef]
22. Feng, S.; Li, H.; Tai, Y.; Huang, J.; Su, Y.; Abramowitz, J.; Zhu, M.X.; Birnbaumer, L.; Wang, Y. Canonical transient receptor potential 3 channels regulate mitochondrial calcium uptake. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 11011–11016. [CrossRef] [PubMed]
23. Harper, M.T.; Londono, J.E.; Quick, K.; Londono, J.C.; Flockerzi, V.; Philipp, S.E.; Birnbaumer, L.; Freichel, M.; Poole, A.W. Transient receptor potential channels function as a coincidence signal detector mediating phosphatidylserine exposure. *Sci. Signal* **2013**, *6*, ra50. [CrossRef] [PubMed]
24. Kistler, A.D.; Singh, G.; Altintas, M.M.; Yu, H.; Fernandez, I.C.; Gu, C.; Wilson, C.; Srivastava, S.K.; Dietrich, A.; Walz, K.; et al. Transient receptor potential channel 6 (TRPC6) protects podocytes during complement-mediated glomerular disease. *J. Biol. Chem.* **2013**, *288*, 36598–36609. [CrossRef]
25. Nakhoul, N.L.; Tu, C.L.; Brown, K.L.; Islam, M.T.; Hodges, A.G.; Abdulnour-Nakhoul, S.M. Calcium-sensing receptor deletion in the mouse esophagus alters barrier function. *Am. J. Physiol. Gastrointest. Liver Physiol.* **2020**, *318*, G144–G161. [CrossRef] [PubMed]
26. Ram, S.; Vizcarra, P.; Whalen, P.; Deng, S.; Painter, C.L.; Jackson-Fisher, A.; Pirie-Shepherd, S.; Xia, X.; Powell, E.L. Pixelwise H-score: A novel digital image analysis-based metric to quantify membrane biomarker expression from immunohistochemistry images. *PLoS ONE* **2021**, *16*, e0245638. [CrossRef] [PubMed]
27. Magaki, S.; Hojat, S.A.; Wei, B.; So, A.; Yong, W.H. An Introduction to the Performance of Immunohistochemistry. *Methods Mol. Biol.* **2019**, *1897*, 289–298. [PubMed]
28. Pillai-Kastoori, L.; Schutz-Geschwender, A.R.; Harford, J.A. A systematic approach to quantitative Western blot analysis. *Anal. Biochem.* **2020**, *593*, 113608. [CrossRef] [PubMed]
29. Taylor, S.C.; Posch, A. The design of a quantitative western blot experiment. *Biomed. Res. Int.* **2014**, *2014*, 361590. [CrossRef] [PubMed]
30. Lang, H.B.; Xie, R.X.; Huang, M.L.; Fang, L.Y.; Tang, Y.B.; Zhang, F. The Effect and Mechanism of TRPC1, 3, and 6 on the Proliferation, Migration, and Lumen Formation of Retinal Vascular Endothelial Cells Induced by High Glucose. *Ophthalmic Res.* **2020**, *63*, 284–294. [CrossRef]
31. Du, Y.; Fu, J.; Yao, L.; Qiao, L.; Liu, N.; Xing, Y.; Xue, X. Altered expression of PPAR-gamma and TRPC in neonatal rats with persistent pulmonary hypertension. *Mol. Med. Rep.* **2017**, *16*, 1117–1124. [CrossRef] [PubMed]
32. Uhlen, M.; Fagerberg, L.; Hallstrom, B.M.; Lindskog, C.; Oksvold, P.; Mardinoglu, A.; Sivertsson, A.; Kampf, C.; Sjostedt, E.; Asplund, A.; et al. Proteomics. Tissue-based map of the human proteome. *Science* **2015**, *347*, 1260419. [CrossRef] [PubMed]
33. Available online: https://www.proteinatlas.org/ENSG00000138741-TRPC3/tissue/parathyroid+gland (accessed on 1 March 2024).
34. Agarwal, S.; Kardam, S.; Chatterjee, P.; Kumar, C.; Boruah, M.; Sharma, M.C.; Tabin, M.; Ramakrishnan, L. CaSR expression in normal parathyroid and PHPT: New insights into pathogenesis from an autopsy-based study. *J. Endocrinol. Investig.* **2022**, *45*, 337–346. [CrossRef]
35. Sengul Aycicek, G.; Aydogan, B.I.; Sahin, M.; Cansiz Ersoz, C.; Sak, S.D.; Baskal, N. Clinical Impact of p27(Kip1) and CaSR Expression on Primary Hyperparathyroidism. *Endocr. Pathol.* **2018**, *29*, 250–258. [CrossRef] [PubMed]

36. Worth, A.L.; Ayrapetyan, M.; Maygarden, S.J.; Li, Z.; Wu, Z.; Agala, C.B.; Kim, L.T. Expression of the Calcium-Sensing Receptor on Normal and Abnormal Parathyroid and Thyroid Tissue. *J. Surg. Res.* **2024**, *293*, 618–624. [CrossRef] [PubMed]
37. Kawata, T.; Imanishi, Y.; Kobayashi, K.; Kenko, T.; Wada, M.; Ishimura, E.; Miki, T.; Nagano, N.; Inaba, M.; Arnold, A.; et al. Relationship between parathyroid calcium-sensing receptor expression and potency of the calcimimetic, cinacalcet, in suppressing parathyroid hormone secretion in an in vivo murine model of primary hyperparathyroidism. *Eur. J. Endocrinol.* **2005**, *153*, 587–594. [CrossRef] [PubMed]

3.2 Publikation 2: “Distribution of TRPC1, TRPC3 and TRPC6 in the human thyroid”

Pathology - Research and Practice 266 (2025) 155796

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Distribution of TRPC1, TRPC3, and TRPC6 in the human thyroid

Emilie Kirstein [a], Coline M. Diebolt [a], Mathias Wagner [b], Alessandro Bozzato [c], Jan M. Federspiel [d], Dirk Schaudien [e], Thomas Tschernig [a], Colya N. Englisch [a,*]

[a] Institute for Anatomy and Cell Biology, Saarland University, Homburg, Saar 66421, Germany
[b] Department of Pathology, Saarland University, Homburg, Saar 66421, Germany
[c] Department of Otorhinolaryngology, Head and Neck Surgery, Saarland University Medical Center, Homburg, Saar 66421, Germany
[d] Institute for Legal Medicine, Saarland University, Homburg, Saar 66421, Germany
[e] Fraunhofer Institute for Toxicology and Experimental Medicine, Hanover 30625, Germany

ARTICLE INFO

Keywords:
TRPC1
TRPC3
TRPC6
Thyroid
Thyrocytes
C-cell
Endocrine
Human
Immunohistochemistry

ABSTRACT

Background: Little is known about the protein expression of the transient receptor potential canonical (TRPC) channels 1, 3, and 6 in the thyroid. Research in human tissue is insufficient. Our aim was to investigate the distribution of TRPC1, 3, and 6 in the healthy human thyroid.

Methods: Healthy samples were collected from seven nitrite pickling salt-ethanol-polyethylene glycol-fixed cadavers and from one patient who had undergone neck surgery (5 males, 3 females; median 81.0, interquartile range 6.5 years). The protein expression profiles of TRPC1, 3, and 6 were assessed using immunohistochemistry with knockout-validated antibodies. A monoclonal calcitonin antibody was used to detect calcitonin-producing C-cells.

Results: All samples were labeled as healthy, displaying age-appropriate signs of degeneration. TRPC1, 3, and 6 immunolabeling in thyrocytes showed irregular staining patterns leaving selected cells with intense staining, some without. The comparison of calcitonin- and TRPC1-, 3-, and 6-immunolabeled slides strongly suggested TRPC1, 3, and 6 expression in C-cells. Connective tissue showed no immunoreactivity.

Conclusions: This is, to the authors' knowledge, the first detailed description of the distribution of these channels in the human thyroid. We conclude that TRPC1, 3, and 6 are expressed in thyrocytes and C-cells of the human thyroid. Further studies are necessary to confirm these small-case-number results and to explore the relevance of these versatile channels in thyroidal health and disease.

1. Introduction

The family of transient receptor potential (TRP) channels constitutes a group of cellular, membrane-bound, non-selective ion channels, distributed across various tissues and cell types in both animal and human organisms [1]. These channels play a pivotal role in physiological processes, acting as essential mediators of sensory signals, signaling pathways, and contributing to various pathophysiological conditions. Notably, they are implicated in the pathogenesis of diseases affecting the cardiovascular, skeletal, renal, and nervous systems [2–4]. Currently, the mammalian TRP-channel family is subclassified into the canonical (TRPC), the melastatin (TRPM), the vanilloid (TRPV), the mucolipin (TRPML), the polycystin (TRPP), the ankyrin (TRPA), and the "no mechanoreceptor potential C" (TRPN) subfamilies [5,6]. The TRPC subfamily comprises seven members (TRPC1–7) of non-selective vastly Ca^{2+}-permeable cation channels [5]. They display a tetrameric structure, in which each monomer or subunit is composed of six transmembrane segments (S1–S6) with variably assembled cytoplasmic domains at each terminus. The S5 and S6 transmembrane segments of each monomer form the Ca^{2+}-permeable cation-pore [2], emphasizing the crucial role of TRPC channels in cellular Ca^{2+}-household and -signaling. They are manifoldly expressed in human organs, influencing a multitude of cellular processes such as transcription factor activation, apoptosis, and cell proliferation [7–10]. TRPC channels can function as receptor-operated Ca^{2+}-entry (ROCE) and/or store-operated Ca^{2+}-entry (SOCE) channels. Activation of the G protein-coupled receptor G_q, promotes phospholipase C (PLC)-mediated release of inositol-triphosphate (IP_3) and diacylglycerol (DAG). DAG can initiate ROCE by stimulating TRPC channels resulting in an increase in cytosolic Ca^{2+}-concentration. IP_3 in turn rather triggers the SOCE pathway by releasing Ca^{2+} from

* Correspondence to: Institute for Anatomy and Cell Biology, Saarland University, Building 61, Kirrberger Strasse, Homburg, Saar 66421, Germany
E-mail address: colya.englisch@uni-saarland.de (C.N. Englisch).

https://doi.org/10.1016/j.prp.2024.155796
Received 20 September 2024; Received in revised form 23 December 2024; Accepted 23 December 2024
Available online 24 December 2024

intracellular stores, such as the endoplasmic reticulum, ultimately activating store-operated channels (SOCs) in the plasma membrane resulting in Ca^{2+}-influx reinforcement [11,12]

Ca^{2+}-signaling also plays an essential role in the thyroid. In addition to the classical thyroid stimulating hormone (TSH)-evoked cyclic adenosine monophosphate (cAMP) pathway, TSH induces Ca^{2+}-signaling through PLC-IP_3 pathways [13]. Of note, also thyroidal cell proliferation and desoxyribonucleic acid (DNA)-synthesis are dependent upon Ca^{2+}-signaling [14,15]. Furthermore, regulation of TSH-receptor and thyroglobulin expression and synthesis are intricately linked to changes in intracellular Ca^{2+}-levels [16,17]. Previous studies have detected ribonucleic acid (RNA) sequences encoding selected TRPC channels in the human thyroid, and provided evidence suggesting their involvement in thyroidal Ca^{2+}-signaling pathways [18,19]. However, a comprehensive histological description of the respective protein expression and distribution is missing. With this in mind, a short summary of the physiology and anatomy of the thyroid is necessary.

Situated in the lower neck, the thyroid is an endocrine gland comprising two lobes connected by an isthmus [20]. It is located anterior to the trachea and inferior to the cricoid cartilage [21] (Fig. 1). The thyroid is critical in following functions [22]. First, the hormone production (triiodothyronine, T3; and tetraiodothyronine, T4) by the thyroid follicular epithelial cells (i.e., thyrocytes), that constitute a major portion of the thyroid tissue. These hormones act on various organs including the heart, lungs, and skeletal muscle [23]. Second, the secretion of the peptide hormone calcitonin by so-called C-cells as a response to increased serum Ca^{2+}-concentration [24]. Aforementioned thyrocytes form a single-layered epithelial layer lining the follicullar colloid in which T3 and T4 are stored (Fig. 1). In contrast, the C-cells, also known as parafollicular cells, constitute a minority. These cells are isolated or grouped within a common basal lamina with the follicular epithelial cells even though without connection to the follicular colloid (Fig. 1). [24]

Asghar and colleagues investigated expression of TRPC RNA in healthy and diseased thyroids using polymerase chain reaction (PCR). Their results suggest a significant involvement of Ca^{2+}-signaling including TRPC channel activity in the progression of thyroid cancer [13,18]. However, the authors had a focus on TRPC1 [18], ultimately disregarding players like TRPC3 and TRPC6 which are nevertheless also attributed a number of roles in various pathophysiological processes [3,4,26]. Moreover, TRPC1 is suggested to form complexes with TRPC3 [27], which in turn is known to build heteromers with TRPC6 [28,29]. This is critical since heteromerization substantially enlarges the spectrum of functions that TRPC tetramers can obtain – in health and disease [30]. In summary, there is need for a more detailed exploration of TRPC channel protein expression in the human thyroid to support further research on potential pharmacological targets [25]. Therefore, the aim of this study was to comprehensively investigate the TRPC1, 3, and 6 protein expression and distribution in the healthy human thyroid.

2. Methods and materials

2.1. Specimens

Thyroid samples were obtained from human adults who decided to donate their bodies to education and science after death and from one who had undergone neck surgery (Table 1). Five of the human tissue samples were obtained from male specimens, three from female. Median age at death or surgery was 81.0 with an interquartile range of 6.5 years.

Body donors had been fixed within 72 hours postmortem by retrograde perfusion with nitrite pickling salt-ethanol polyethylene glycol fixation (NEP) through the femoral artery according to Weigner's protocol [31]. Afterwards, immersion fixation was applied by placing the bodies in 3 % formalin and NEP-solution for up to 3 months until completion of the fixation process. Thyroid samples were obtained from the right and left lobe using punch biopsies during dissection courses at the anatomical institute.

The surgery sample had been obtained from the right lobe in the context of parathyroid adenoma surgery. Fixation was performed within the surgical schedule using 4 % phosphate-buffered formalin.

The fixated tissue samples were then embedded in paraffin as described before [32]. From that, 4 μm thick sections were prepared using a microtome and drawn onto microscopic slides.

Table 1
Overview of the samples (age [at death or surgery], sex, diagnosis/cause of death, tissue origin).

Number	Age	Sex	Diagnosis/Cause of death	Tissue Origin
1	81	Male	Parathyroid adenoma	Surgery
2	76	Female	Embolism	Body donor
3	75	Male	Septic shock, pneumonia	Body donor
4	82	Male	Kidney failure, metastatic prostate cancer	Body donor
5	83	Female	Cardiogenic shock	Body donor
6	81	Female	Cardiopulmonary insufficiency	Body donor
7	90	Male	Cardioembolic event	Body donor
8	81	Male	Multiple organ failure	Body donor

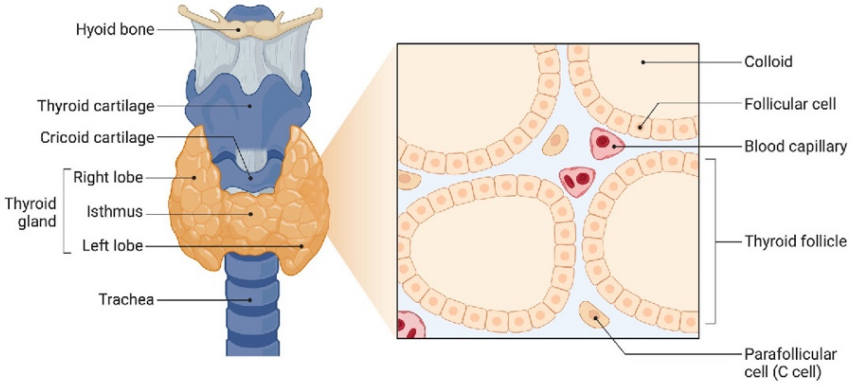


Fig. 1. Anatomy and histology of the thyroid. Follicular cells (i.e., thyrocytes) make the largest part of the tissue lining the thyroidal follicle. Calcitonin-producing parafollicular or C-cells are isolated or grouped around the follicles. Follicles are surrounded by tissue and close-meshed capillary network. Created with BioRender (Agreement number: WU267U400D).

All investigations were performed anonymously and in accordance with the guidelines of the Declaration of Helsinki. This study was approved by the Ethics Committee of the Saarland Medical Association (vote numbers: 163/20, and 130/21). Informed consent was obtained from the patient and the body donors.

2.2. Hematoxylin & eosin staining

Hematoxylin & eosin (H&E) staining was performed using standardized techniques [33]. Briefly summarized, samples were deparaffinized in 100 % Xylol for 15 min and rehydrated in decreasing concentrated ethanol solutions (2 × 100 %, 1 × 90 %, and 1 × 80 %, 5 min each). After 8-min-long staining in Ehrlich hematoxylin (C. Roth, Karlsruhe, Germany), the samples were washed in distillated water and blued in fluent tap water for 12 min. Staining in 0.1 % eosin followed (Central Chemical Storage, Saarland University, Saarbrücken, Germany). Finally, the samples were dehydrated using increasing ethanol concentration series (1 × 90 % and 2 × 100 % ethanol, and 3 × 100 % Xylol, 5 min each).

2.3. Immunohistochemistry

The polyclonal knockout-validated rabbit TRPC1 (ACC-010, Alomone Labs, Jerusalem, Israel), TRPC3 (ACC-016, Alomone Labs, Jerusalem, Israel), and TRPC6 antibodies (ACC-017, Alomone Labs, Jerusalem, Israel) were used.

Samples were incubated in citrate buffer in a heating incubator at 95°C for 60 min. Then, the primary antibody which was diluted 1:50 in phosphate-buffered saline was applied. For negative controls, a 1:500 diluted rabbit serum was used. After overnight incubation, a secondary antibody (horseradish peroxidase, goat anti-rabbit, A10547, Invitrogen AG, Carlsbad, CA, USA) and a chromogen (diaminobenzidine tetrachloride [DAB], SK-4103, Vector Laboratories, Burlingame, CA, USA) were applied. DAB incubation time was set at 5 min. Nuclear counterstaining with Ehrlich hematoxylin followed (C. Roth, Karlsruhe, Germany).

According to the manufacturer's information, the TRPC1, 3, and 6 antibodies are knockout-validated and generated to recognize the corresponding channels in mouse, rat, and human tissues [34–37]. In detail, the TRPC1 antibody detects the amino acid residues 557–571 of human TRPC1 (Peptide QLYDKGYTSKEQKDC), the TRPC3 antibody the residues 822–835 of murine TRPC3 (Peptide HKLSEKLNPSVLRC), and the TRPC6 antibody the residues 24–38 of murine TRPC6 (Peptide [C] RRNESQDYLLMDELG).

Additionally, immunohistochemistry was performed using a monoclonal rabbit calcitonin antibody generated to recognize the human calcitonin 1–32 peptide (SP17, Invitrogen, Thermo Fisher Scientific, MA, USA). Immunohistochemistry was performed as aforementioned for TRPC1, 3, and 6 except for a longer DAB incubation time of 12 min.

2.3.1. Histological identification of C-cells

Distinguishing C-cells from thyrocytes can be challenging [38]. The designation "C-cells" is derived from the term "clear cells," which refers to their low affinity for certain histological staining methods. Thyrocytes line the thyroidal follicle. Their size depends on their activity fluctuating from flat or inactive over cuboidal to columnar or highly active [39]. In an H&E stain normal C-cells cannot be delineated with complete certainty. Depending on cutting direction, they can appear like thyrocytes. C-cells can be round, polygonal or spindle shaped with a larger and lightened nucleus, in comparison to thyrocytes. The name "parafollicular cells" was introduced 1932 and widely used since, which can be misleading considering that less than 1 % of human C-cells are parafollicular [38]. As aforementioned, they often occur in the cellular lining of the follicles, though remaining separated by thyrocytes from the follicular content also called colloid [39].

2.4. Visualization

Histological slides were digitalized using the Nano Zoomer S210 (Hamamatsu, Japan). Microphotographs were taken using the image viewing software NDP.view2 from Hamamatsu (U12388–01, Hamamatsu, Japan). Representative microphotographs for the cohort were mostly obtained from the surgical specimen to enhance quality of illustration.

3. Results

3.1. Overview

All samples were tumor free and age-appropriate, as evaluated and labeled by trained pathologists. All cadaveric samples showed varying degree of autolysis but mostly intact tissue. The following description refers to all samples, unless differently mentioned.

H&E staining displayed a physiological lobar parenchymatous architecture and subarchitecture (Fig. 2). Thyrocytes and C-cells were identified according to the aforementioned criteria (Fig. 2C and D).

Immunohistochemical calcitonin staining displayed a sparse pattern of isolated and grouped calcitonin-produding C-cells (Fig. 3A and B). Follicular epithelial cells and connective tissue were immunolabeling-deficient. Negative control of the calcitonin antibody displayed no DAB-specific coloration (Fig. 3C).

3.1.1. TRPC1

TRPC1 immunoreactivity was detected in thyrocytes (Fig. 4). Throughout all samples, thyrocyte staining was heterogenous with respect to coloration intensity (Fig. 4). Few thyrocytes displayed no staining at all (Fig. 4B). C-cells displayed a homogenous immunoreactivity consistent with TRPC1 protein expression (Fig. 4C). In contrast, connective tissue was not immunolabeled. Negative control of the TRPC1 antibody displayed no DAB-specific coloration (Fig. 3D).

3.1.2. TRPC3

TRPC3 immunoreactivity was detected in thyrocytes (Fig. 5). Throughout all samples, thyrocyte staining was heterogenous with respect to coloration intensity (Fig. 5). Few thyrocytes displayed no staining at all (Fig. 5B). C-cells displayed a homogenous immunoreactive signal consistent with TRPC3 protein expression (Fig. 5C). In contrast, connective tissue was not immunolabeled. Negative control of the TRPC3 antibody displayed no DAB-specific coloration (Fig. 3E).

3.1.3. TRPC6

TRPC6 immunoreactivity was detected in thyrocytes (Fig. 6). Throughout all samples, thyrocyte staining was heterogenous with respect to coloration intensity (Fig. 6). Few thyrocytes displayed no staining at all (Fig. 6B and C). C-cells displayed a homogenous immunoreactive signal consistent with TRPC6 protein expression (Fig. 6C and D). In contrast, connective tissue was not immunolabeled. Negative control of the TRPC6 antibody displayed no DAB-specific coloration (Fig. 3F).

4. Discussion

4.1. Overview

The aim of this study was to investigate protein expression and distribution of TRPC1, 3, and 6 in the healthy human thyroid. All analyzed tissue samples exhibited extensive TRPC1, 3, and 6 protein expression. Immunohistochemistry revealed heterogenous staining of thyrocytes that was comparable in all specimens. Protein expression was also detected in C-cells.

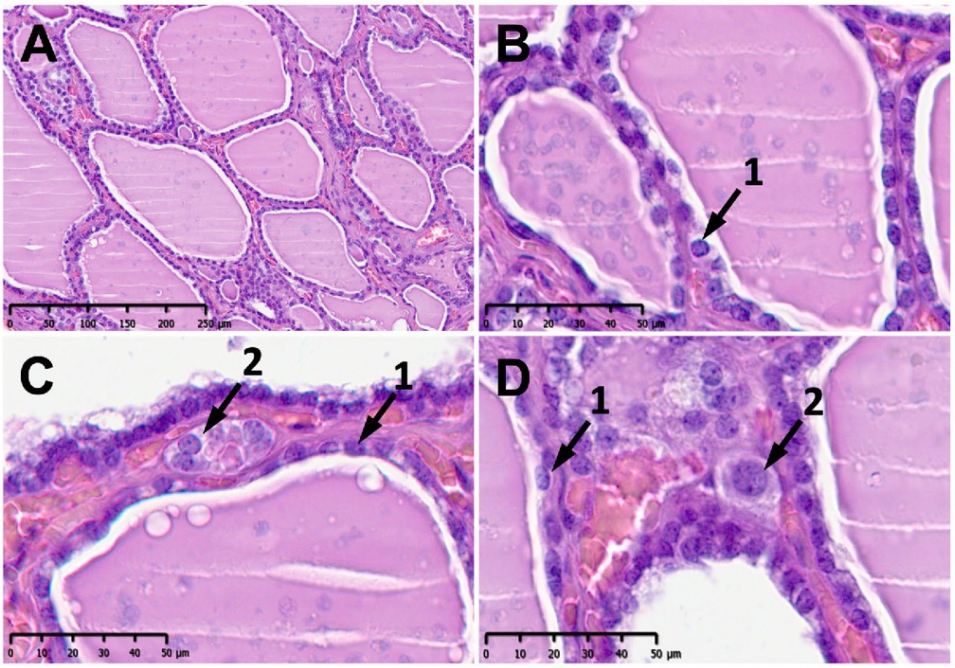


Fig. 2. Hematoxylin & eosin staining of the thyroid. Numerous colloid structures enclosed by thyrocytes and thyroid stroma (A; 10 × software magnification). Thyrocytes in higher magnification (B, C, and D; 40 × magnification). Grouped and isolated C-cells (C and D; 40 × magnification). Thyrocytes (1), C-cells (2).

4.2. TRPC1, 3, and 6 in thyrocytes

Whereas the relevance of TRPC1, 3, and 6 in thyroidal physiology and pathophysiology is not yet elucidated, the importance of Ca^{2+}-signaling is accepted [13,14,16,17]. TRPC1, 3, and 6 are integral in Ca^{2+}-signaling, which governs essential cancer-related processes such as migration, invasion, and proliferation [13,25]. The RNA of TRPC1, 3, and 6 is expressed in normal thyroid tissue [18]. The ability of these channels to regulate intracellular Ca^{2+}-levels makes them promising candidates for targeted therapies aimed at controlling thyroid cancer progression. Concretely, Asghar *et al.* demonstrated a significant role for TRPC1 in invasion, migration, and proliferation in human thyroid cancer cells [18]. Nevertheless, TRPC3 and 6 are important modulators of these cellular activities [25]. For instance, TRPC6 is known to play a central role in migration and invasion [40–43], whereas TRPC3 has been shown to influence cancer cells in several tissues through its role in calcium signaling and its interactions with other signaling pathways [44,45].

In experiments with rat thyroid cells exclusively expressing the TRPC2, a crucial role of this channel in various physiological processes was observed [15,46,47]. However, the TRPC2 protein is not expressed in humans, *Trpc2* being a pseudogene [48]. Still, to the authors' knowledge, a histological analysis of the expression of the remaining TRPC channels in human thyroid tissue has not been part of previous investigations. Asghar *et al.* presented in their publication a characterization of TRPC channels using molecular genetic methods [18]. Though it must be considered, as also mentioned by themselves, that the tissue might contain other cells expressing TRPC channels apart of thyrocytes [18]. Also, the detection of nucleic acids doesn't necessarily signify corresponding protein expression [49,50]. With our investigations we provide clear evidence that thyrocytes express the TRPC1, 3, and 6 proteins. Notably, TRPC channels have been implicated in other endocrine organs [51–53]. For instance, we recently provided evidence showing TRPC3 and TRPC6 protein expression in the human pancreas and parathyroid glands [54,55]. Summarizing, for the first time the TRPC1, 3, and 6 protein expression in human thyroidal tissue was evidenced. Further translational research is warranted to study the involvement of these channels in the cellular function of healthy and diseased thyrocytes, which is yet unclear.

4.3. TRPC1, 3, and 6 in C-cells

In contrast to thyrocytes, there are no studies published describing the expression of TRPC channels in C-cells to the authors' knowledge. This study provides evidence suggesting the protein expression of TRPC1, 3, and 6 in C-cells. As aforementioned identification of C-cells in light microscopy can be challenging due to their rarity [38] and uneven distribution [56]. To confirm our morphological C-cell identification, we performed calcitonin immunolabeling. Here, several stained cells, i.e., calcitonin producing C-cells, were detected. Although sections were sequentially prepared, no subcellular match was found with the TRPC1, 3, and 6 staining. Nevertheless, the morphology of calcitonin-immunostained cells supports the validity of our C-cell identification based on aforementioned descriptions in the TRPC1, 3, and 6 immunolabeled slides. Since studies have shown that the calcitonin peptide content displayed a strong correlation with the morphology of calcitonin-producing cells in the normal thyroid [57], it can be assumed that the calcitonin immunoreactive cells likely were active C-cells.

However, it must be considered that our samples were from older patients and that the number of calcitonin immunoreactive cells in elderly can greatly vary [58]. Therefore, the presented results cannot be translated one-to-one to younger specimens.

Altogether, these results warrant further research with larger case numbers also involving younger individuals. Nevertheless our investigations strongly indicate TRPC1, 3, and 6 protein expression in C-

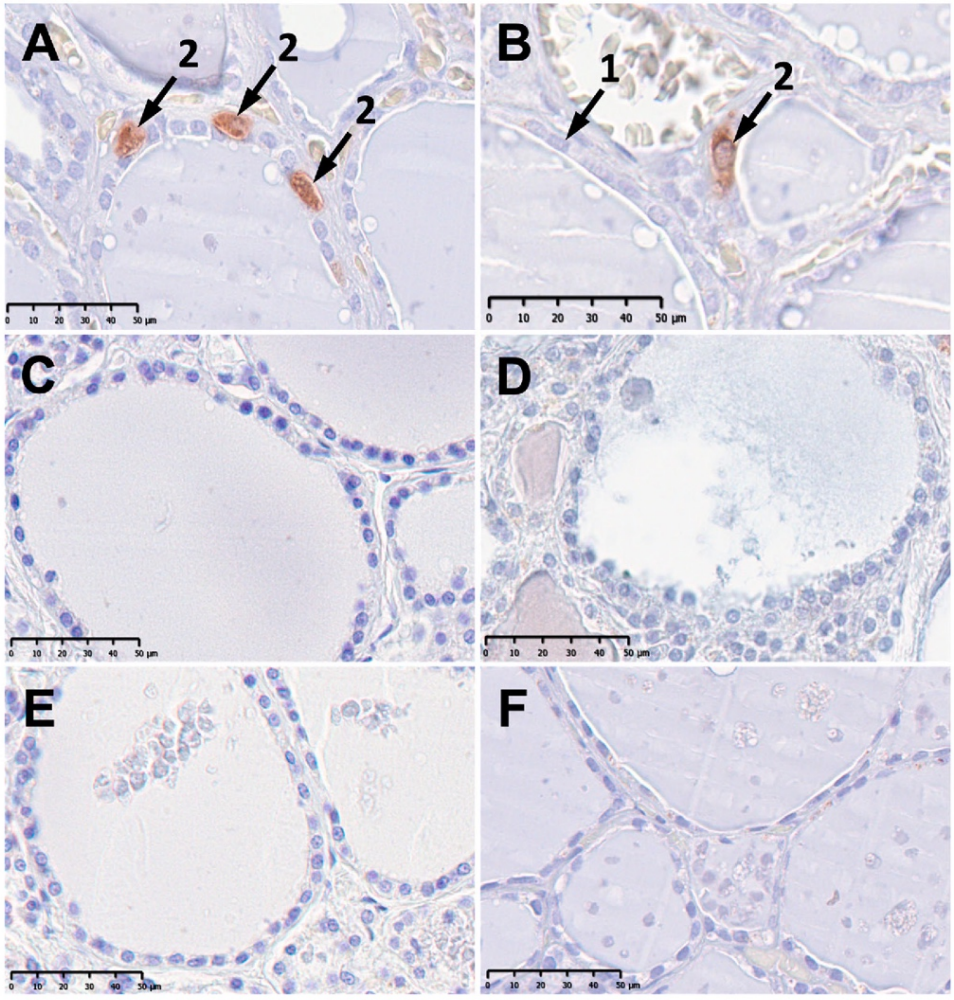


Fig. 3. Calcitonin expression in the human thyroid (A and B). Immunostaining with the calcitonin antibody in C-cells. Thyrocytes (1) and C-cells (2) are displayed (A and B; 40 × magnification). Negative control staining of the calcitonin (C; 40 × magnification), TRPC1 (D; 40 × magnification), TRPC3 (E; 40 × magnification), and TRPC6 (F; 40 × magnification) antibodies showed no DAB coloration.

cells. Considering that significant pathologies such as medullary thyroid carcinoma originate from C-cells [38] and that TRPC channels are involved in the development of cancer [25] further investigations could yield interesting results. In this context, targeted therapies hitting medullary thyroid carcinoma drivers such as the tyrosine kinase receptors VEGFR (vascular endothelial growth factor receptor), EGFR (epidermal growth factor receptor), and MET (mesenchymal-epithelial transition factor) yield promising results [59,60]. As a matter of fact, in the context of renal cell carcinoma a link between TRPC6 and MET has been observed as summarized in [4], foregrounding the increasing translational interest in studying TRPC channels and their potential involvement in Ca^{2+}-signaling-linked tumorigenesis and/or tumor progression.

4.4. Methodology and limitations

Immunohistochemistry is a highly specific method [61]. It can detect small amounts of protein. In connection with the histological evaluation, it not only allows for evaluation of corresponding protein expression but also protein distribution in a histological context. For this purpose, its superiority to molecular genetic methods is obvious [62]. A serious drawback is the lacking option of protein quantification. Furthermore, immunohistochemistry doesn't provide any information about the channels integrity or activity. Besides, it must be addressed that the method of immunohistochemistry is a multi-stage process and variability can be introduced at any stage leading to a more difficult reproducibility of results. Tissue handling from body donors that were fixated postmortem can show, for example, protein degradation [63] or tissue liquification [64]. Importantly, the surgical sample showed similar results, ultimately indicating the transferability of the findings [65]. Furthermore, tissue processing can lead to loss of information of its natural state. While formalin as fixative method is widely used, cross-linking of proteins as significant characteristic can interfere with the antigens' ability to react with the primary antibody [66,67]. In the

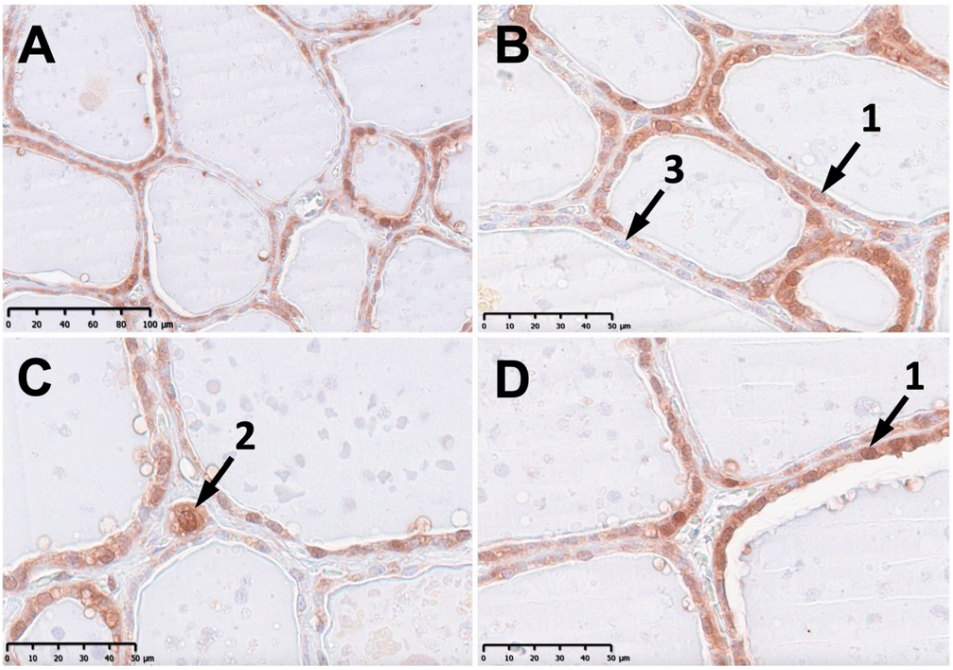


Fig. 4. TRPC1 protein expression in the human thyroid. Overview (A; 20 × magnification) and higher magnification microphotographs (B, C, and D; 40 × magnification). Immunostaining-positive thyrocytes (1), C-cells (2), and immunostaining-deficient thyrocytes (3) are displayed.

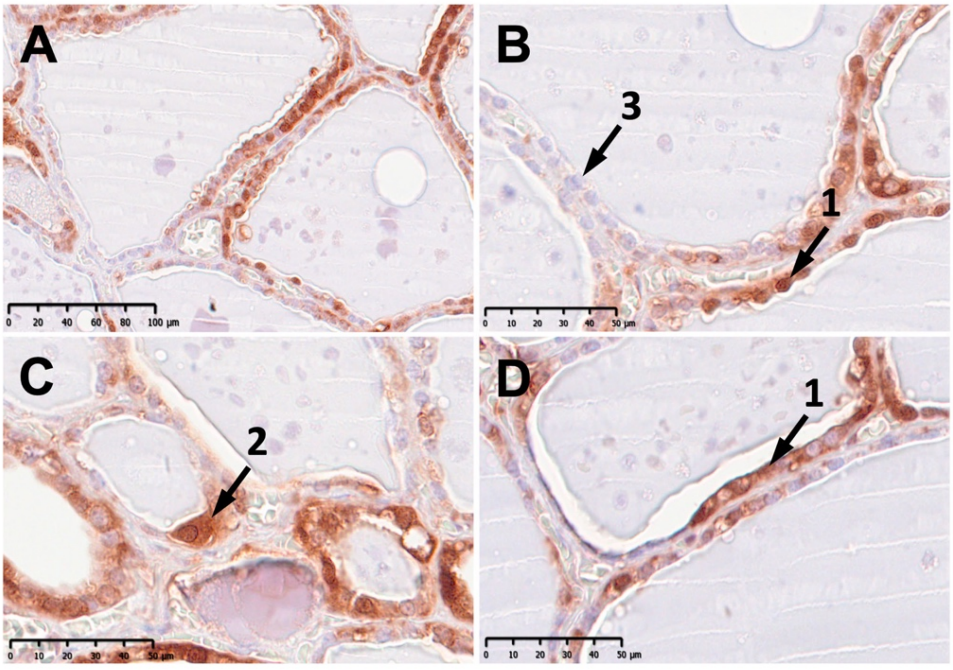


Fig. 5. TRPC3 protein expression in the human thyroid. Overview (A; 20 × magnification) and higher magnification microphotographs (B, C, and D; 40 × magnification). Immunostaining-positive thyrocytes (1), C-cells (2), and immunostaining-deficient thyrocytes (3) are displayed.

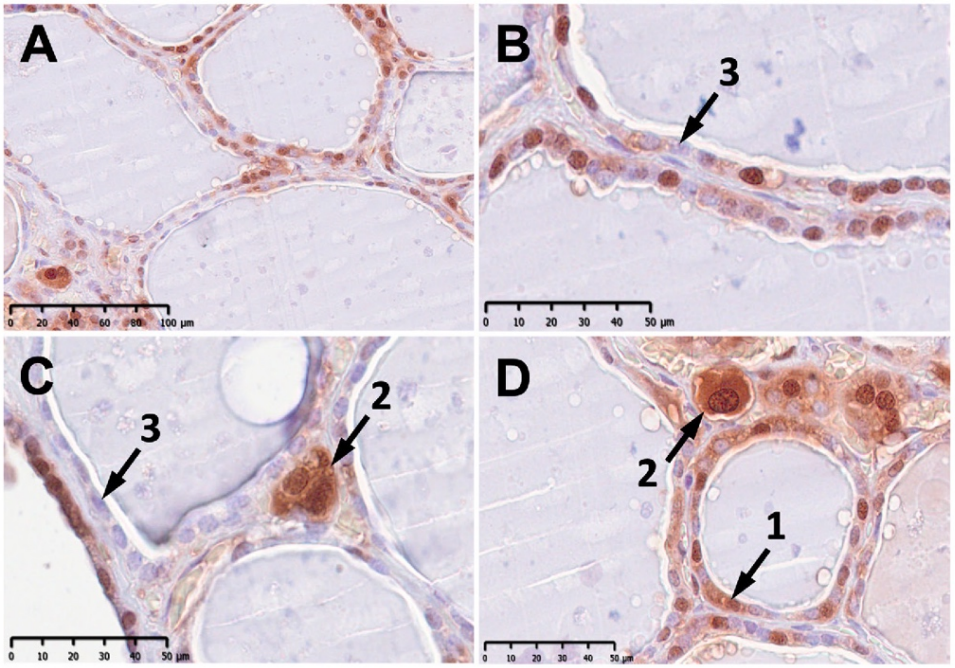


Fig. 6. TRPC6 protein expression in the human thyroid. Overview (A; 20 × magnification) and higher magnification microphotographs (B, C, and D; 40 × magnification). Immunostaining-positive thyrocytes (1), C-cells (2), and immunostaining-deficient thyrocytes (3) are displayed.

process of antigen retrieval, the conditions of heating and the pH of the buffer solution during the heating process can influence the effectiveness of the antigen retrieval [62]. Even though, our antigen retrieval was performed using standardized techniques, one cannot exclude the possibility of undetected antigens. Moreover, no studies showing knockout validation for the used monoclonal calcitonin antibody exist (SP17, Invitrogen, Thermo Fisher Scientific, MA, USA). Uncertainty with regard to potential lack in specificity is therefore given, although the staining pattern was very plausible to reflect the C-cell distribution.

The heterogenous staining pattern described in TRPC1, 3, and 6 immunohistochemistry could be due to the above-described limitations. With regard to the surgery sample, one cannot exclude the possibility that neighbored thyroid tissue could have been affected on a biochemical level from abnormal changes due to parathyroid adenoma, even though the risk remains small as illustrated by previous studies, that investigated TRPC channels using tumor nephrectomy tissue [36,68]. As aforementioned, form and structure of thyrocytes can vary depending on the state of activity. A correlation between thyrocyte activity and staining intensity is possible [69,70]. As a matter of fact, highly prismatic and columnar cellular shape frequently corresponded to increased staining intensity in all immunoreactive stainings with TRPC1, 3 and 6 antibodies, leaving this open for further interpretation. Finally, it also needs to be kept in mind that staining intensity doesn't necessarily correlate with protein expression level.

This study analyzed a small case number. Thus, inter- and intra-individual variances could have been missed. Additionally, all samples were obtained from older patients. The results described in this study do not mirror the conditions in younger specimens. Further investigations are necessary to determine the translationability of our results.

5. Conclusions

The TRPC1, 3 and 6 proteins are expressed in thyrocytes and C-cells of the human thyroid. Further studies are needed to investigate the physiological function of these channels in thyrocytes and C-cells, and to assess their involvement in cell-derived pathologies.

Funding

This study was not funded by any exterior sources.

Ethical statement

All investigations were implemented anonymously, approved by the Ethics Committee of the Saarland Medical Association (163/20, 130/21), and performed in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from the patient and the body donors.

CRediT authorship contribution statement

Mathias Wagner: Writing – review & editing, Resources. **Coline M. Diebolt:** Writing – review & editing, Methodology, Investigation. **Emilie Kirstein:** Writing – original draft, Visualization, Methodology, Investigation, Formal analysis, Data curation. **Jan M. Federspiel:** Writing – review & editing, Formal analysis. **Colya N. Englisch:** Writing – review & editing, Visualization, Supervision, Conceptualization. **Thomas Tschernig:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Conceptualization. **Alessandro Bozzato:** Writing – review & editing, Resources. **Dirk Schaudien:** Formal analysis, Writing – review & editing.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

The authors wish to sincerely thank those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially improve patient care and increase mankind`s overall knowledge. Therefore, these donors and their families deserve our highest gratitude. Additionally, we thank Anja Beckmann as well as Martin Jung and Carola Meier for material support.

References

[1] K. Venkatachalam, C. Montell, TRP channels, Annu Rev. Biochem 76 (2007) 387–417, https://doi.org/10.1146/annurev.biochem.75.103004.142819.
[2] Y. Kaneko, A. Szallasi, Transient receptor potential (<scp>TRP</scp>) channels: a clinical perspective, Br. J. Pharm. 171 (2014) 2474–2507, https://doi.org/10.1111/bph.12414.
[3] C.N. Englisch, C.M. Diebolt, D. Schaudien, G.G. Klamminger, E. Kirstein, K. Junker, M. Wagner, T. Tschernig, Proximal tubular TRPC3 immunostaining is reduced in human nephrocalcinosis, Lab. Investig. 104 (2024) 102109, https://doi.org/10.1016/j.labinv.2024.102109.
[4] C.N. Englisch, F. Paulsen, T. Tschernig, TRPC channels in the physiology and pathophysiology of the renal tubular system: what do we know? Int J. Mol. Sci. 24 (2022) 181, https://doi.org/10.3390/ijms24010181.
[5] H. Li, TRP Channel Classif. (2017) 1–8, https://doi.org/10.1007/978-94-024-1088-4_1.
[6] B. Nilius, G. Owsianik, The transient receptor potential family of ion channels, Genome Biol. 12 (2011) 218, https://doi.org/10.1186/gb-2011-12-3-218.
[7] S.F. Pedersen, G. Owsianik, B. Nilius, TRP channels: an overview, Cell Calcium 38 (2005) 233–252, https://doi.org/10.1016/j.ceca.2005.06.028.
[8] X. Liu, K.T. Cheng, B.C. Bandyopadhyay, B. Pani, A. Dietrich, B.C. Paria, W. D. Swaim, D. Beech, E. Yildrim, B.B. Singh, L. Birnbaumer, I.S. Ambudkar, Attenuation of store-operated Ca $^{2+}$ current impairs salivary gland fluid secretion in TRPC1(−/−) mice, Proc. Natl. Acad. Sci. 104 (2007) 17542–17547, https://doi.org/10.1073/pnas.0701254104.
[9] H.L. Ong, L.B. de Souza, K.T. Cheng, I.S. Ambudkar, Physiol. Funct. Regul. TRPC Channels (2014) 1005–1034, https://doi.org/10.1007/978-3-319-05161-1_12.
[10] C.N. Englisch, J. Steinhäuser, S. Wemmert, M. Jung, J. Gawlitza, G. Wenzel, B. Schick, T. Tschernig, Immunohistochemistry reveals TRPC channels in the human hearing organ—a novel CT-guided approach to the cochlea, Int J. Mol. Sci. 24 (2023) 9290, https://doi.org/10.3390/ijms24119290.
[11] C. Fan, W. Choi, W. Sun, J. Du, W. Lü, Structure of the human lipid-gated cation channel TRPC3, Elife 7 (2018), https://doi.org/10.7554/eLife.36852.
[12] J. Abramowitz, L. Birnbaumer, Physiology and pathophysiology of canonical transient receptor potential channels, FASEB J. 23 (2009) 297–328, https://doi.org/10.1096/fj.08-119495.
[13] M.Y. Asghar, T. Lassila, K. Törnquist, Calcium signaling in the thyroid: friend and foe, Cancers 13 (2021) 1994, https://doi.org/10.3390/cancers13091994.
[14] K. Takada, N. Amino, H. Tada, K. Miyai, Relationship between proliferation and cell cycle-dependent Ca2+ influx induced by a combination of thyrotropin and insulin-like growth factor-I in rat thyroid cells, J. Clin. Investig. 86 (1990) 1548–1555, https://doi.org/10.1172/JCI114874.
[15] P. Sukumaran, C. Löf, I. Pulli, K. Kemppainen, T. Viitanen, K. Törnquist, Significance of the transient receptor potential canonical 2 (TRPC2) channel in the regulation of rat thyroid FRTL-5 cell proliferation, migration, adhesion and invasion, Mol. Cell Endocrinol. 374 (2013) 10–21, https://doi.org/10.1016/j.mce.2013.03.026.
[16] M. Saji, S. Ikuyama, T. Akamizu, L.D. Kohn, Increases in cytosolic Ca+ + down regulate thyrotropin receptor gene expression by a mechanism different from the cAMP signal, Biochem Biophys. Res Commun. 176 (1991) 94–101, https://doi.org/10.1016/0006-291X(91)90894-D.
[17] B. Di Jeso, R. Pereira, E. Consiglio, S. Formisano, J. Satrustegui, I.V. Sandoval, Demonstration of a Ca $^{2+}$ requirement for thyroglobulin dimerization and export to the golgi complex, Eur. J. Biochem 252 (1998) 583–590, https://doi.org/10.1046/j.1432-1327.1998.2520583.x.
[18] M.Y. Asghar, M. Magnusson, K. Kemppainen, P. Sukumaran, C. Löf, I. Pulli, V. Kalhori, K. Törnquist, Transient receptor potential canonical 1 (TRPC1) channels as regulators of sphingolipid and VEGF receptor expression, J. Biol. Chem. 290 (2015) 16116–16131, https://doi.org/10.1074/jbc.M115.643668.
[19] D. Choukair, B. Eberle, P. Vick, P. Hermanns, B. Weiss, N. Paramasivam, M. Schlesner, K. Lornsen, R. Roeth, C. Klutmann, J. Kreis, G.F. Hoffmann, J. Pohlenz, G.A. Rappold, M. Bettendorf, Identification of transient receptor potential channel 4-associated protein as a novel candidate gene causing congenital primary hypothyroidism, Horm. Res Paediatr. 93 (2020) 16–29, https://doi.org/10.1159/000507114.
[20] L.A. Loevner, Imaging of the thyroid gland, Semin. Ultrasound, CT MRI 17 (1996) 539–562, https://doi.org/10.1016/S0887-2171(96)90003-7.
[21] E. Allen, A. Fingeret, Anatomy, Head and Neck, Thyroid, 2023.
[22] S. Hiller-Sturmhöfel, A. Bartke, The endocrine system: an overview, Alcohol Health Res World 22 (1998) 153–164.
[23] M. Armstrong, E. Asuka, A. Fingeret, Physiology, Thyroid Function, 2023.
[24] A. Srinivasan, F.K. Wong, D. Karponis, Calcitonin: a useful old friend, J. Musculoskelet. Neuron Inter. 20 (2020) 600–609.
[25] M.Y. Asghar, K. Törnquist, Transient receptor potential canonical (trpc) channels as modulators of migration and invasion, Int J. Mol. Sci. 21 (2020) 1739, https://doi.org/10.3390/ijms21051739.
[26] P. Eder, K. Groschner, TRPC3/6/7: Topical aspects of biophysics and pathophysiology, Channels 2 (2008) 94–99, https://doi.org/10.4161/chan.2.2.6015.
[27] X. Liu, B.C. Bandyopadhyay, B.B. Singh, K. Groschner, I.S. Ambudkar, Molecular analysis of a store-operated and 2-acetyl-sn-glycerol-sensitive non-selective cation channel, J. Biol. Chem. 280 (2005) 21600–21606, https://doi.org/10.1074/jbc.C400492200.
[28] I. Jardin, L.J. Gómez, G.M. Salido, J.A. Rosado, Dynamic interaction of hTRPC6 with the Orai1–STIM1 complex or hTRPC3 mediates its role in capacitative or non-capacitative Ca2+ entry pathways, Biochem. J. 420 (2009) 267–277, https://doi.org/10.1042/BJ20082179.
[29] T. Hofmann, M. Schaefer, G. Schultz, T. Gudermann, Subunit composition of mammalian transient receptor potential channels in living cells, Proc. Natl. Acad. Sci. 99 (2002) 7461–7466, https://doi.org/10.1073/pnas.102596199.
[30] S. Oda, T. Numaga-Tomita, N. Kitajima, T. Toyama, E. Harada, T. Shimauchi, A. Nishimura, T. Ishikawa, Y. Kumagai, L. Birnbaumer, M. Nishida, TRPC6 counteracts TRPC3-Nox2 protein complex leading to attenuation of hyperglycemia-induced heart failure in mice, Sci. Rep. 7 (2017) 7511, https://doi.org/10.1038/s41598-017-07903-4.
[31] R. Klopfleisch, M. von Deetzen, A.Th Weiss, J. Weigner, F. Weigner, J. Plendl, A. D. Gruber, Weigners fixative–an alternative to formalin fixation for histology with improved preservation of nucleic acids, Vet. Pathol. 50 (2013) 191–199, https://doi.org/10.1177/0300985812441031.
[32] C.M. Diebolt, D. Schaudien, K. Junker, G. Krasteva-Christ, T. Tschernig, C. N. Englisch, New insights in the renal distribution profile of TRPC3 – Of mice and men, Ann. Anat. - Anat. Anz. 252 (2024) 152192, https://doi.org/10.1016/j.aanat.2023.152192.
[33] R.D. Cardiff, C.H. Miller, R.J. Munn, Manual hematoxylin and eosin staining of mouse tissue sections, pdb.prot073411, Cold Spring Harb. Protoc. 2014 (2014), https://doi.org/10.1101/pdb.prot073411.
[34] W. Xia, Q. Wang, S. Lin, Y. Wang, J. Zhang, H. Wang, X. Yang, Y. Hu, H. Liang, Y. Lu, Z. Zhu, D. Liu, A high-salt diet promotes hypertrophic scarring through TRPC3-mediated mitochondrial Ca2+ homeostasis dysfunction, Heliyon 9 (2023) e18629, https://doi.org/10.1016/j.heliyon.2023.e18629.
[35] M.T. Harper, J.E.C. Londoño, K. Quick, J.C. Londoño, V. Flockerzi, S.E. Philipp, L. Birnbaumer, M. Freichel, A.W. Poole, Transient receptor potential channels function as a coincidence signal detector mediating phosphatidylserine exposure, Sci. Signal 6 (2013), https://doi.org/10.1126/scisignal.2003701.
[36] A.D. Kistler, G. Singh, M.M. Altintas, H. Yu, I.C. Fernandez, C. Gu, C. Wilson, S. K. Srivastava, A. Dietrich, K. Walz, D. Kerjaschki, P. Ruiz, S. Dryer, S. Sever, A. K. Dinda, C. Faul, J. Reiser, Transient receptor potential channel 6 (trpc6) protects podocytes during complement-mediated glomerular disease, J. Biol. Chem. 288 (2013) 36598–36609, https://doi.org/10.1074/jbc.M113.488122.
[37] Y. Sun, H. Zhang, S. Selvaraj, P. Sukumaran, S. Lei, L. Birnbaumer, B.B. Singh, Inhibition of l-type Ca $^{2+}$ channels by trpc1-stim1 complex is essential for the protection of dopaminergic neurons, J. Neurosci. 37 (2017) 3364–3377, https://doi.org/10.1523/JNEUROSCI.3010-16.2017.
[38] S. Ting, S. Synoracki, K.W. Schmid, Die C-Zellen der Schilddrüse und ihre Pathologie, Pathologe 36 (2015) 246–253, https://doi.org/10.1007/s00292-015-0019-x.
[39] Y.S. Khan, A. Farhana, Histology, Thyroid Gland, 2023.
[40] S. Chigurupati, R. Venkataraman, D. Barrera, A. Naganathan, M. Madan, L. Paul, J. V. Pattisapu, G.A. Kyriazis, K. Sugaya, S. Bushnev, J.D. Lathia, J.N. Rich, S.L. Chan, Receptor channel TRPC6 is a key mediator of notch-driven glioblastoma growth and invasiveness, Cancer Res 70 (2010) 418–427, https://doi.org/10.1158/0008-5472.CAN-09-2654.
[41] S. Thebault, M. Flourakis, K. Vanoverberghe, F. Vandermoere, M. Roudbaraki, V. Lehen'kyi, C. Slomianny, B. Beck, P. Mariot, J.-L. Bonnal, B. Mauroy, Y. Shuba, T. Capiod, R. Skryma, N. Prevarskaya, Differential role of transient receptor potential channels in Ca2+ entry and proliferation of prostate cancer epithelial cells, Cancer Res 66 (2006) 2038–2047, https://doi.org/10.1158/0008-5472.CAN-05-0376.
[42] R. Diez-Bello, I. Jardin, J.J. Lopez, M. El Haouari, J. Ortega-Vidal, J. Altarejos, G. M. Salido, S. Salido, J.A. Rosado, (−)-Oleocanthal inhibits proliferation and migration by modulating Ca2+ entry through TRPC6 in breast cancer cells, Biochim. Et. Biophys. Acta (BBA) - Mol. Cell Res. 1866 (2019) 474–485, https://doi.org/10.1016/j.bbamcr.2018.10.010.
[43] N. Prevarskaya, R. Skryma, Y. Shuba, Ion channels in cancer: are cancer hallmarks oncochannelopathies? Physiol. Rev. 98 (2018) 559–621, https://doi.org/10.1152/physrev.00044.2016.
[44] K. Oda, M. Umemura, R. Nakakaji, R. Tanaka, I. Sato, A. Nagasako, C. Oyamada, E. Baljinnyam, M. Katsumata, L.-H. Xie, M. Narikawa, Y. Yamaguchi, T. Akimoto, M. Ohtake, T. Fujita, U. Yokoyama, K. Iwatsubo, M. Aihara, Y. Ishikawa, Transient receptor potential cation 3 channel regulates melanoma proliferation and migration, J. Physiol. Sci. 67 (2017) 497–505, https://doi.org/10.1007/s12576-016-0480-1.
[45] H. Storck, B. Hild, S. Schimmelpfennig, S. Sargin, N. Nielsen, A. Zaccagnino, T. Budde, I. Novak, H. Kalthoff, A. Schwab, Ion channels in control of pancreatic stellate cell migration, Oncotarget 8 (2017) 769–784, https://doi.org/10.18632/oncotarget.13647.
[46] C. Löf, P. Sukumaran, T. Viitanen, M. Vainio, K. Kemppainen, I. Pulli, J. Näsman, J. P. Kukkonen, K. Törnquist, Communication between the calcium and cAMP

pathways regulate the expression of the tsh receptor: trpc2 in the center of action, Mol. Endocrinol. 26 (2012) 2046–2057, https://doi.org/10.1210/me.2012-1171.
[47] T.M. Viitanen, P. Sukumaran, C. Löf, K. Törnquist, Functional coupling of TRPC2 cation channels and the calcium-activated anion channels in rat thyroid cells: implications for iodide homeostasis, J. Cell Physiol. 228 (2013) 814–823, https://doi.org/10.1002/jcp.24230.
[48] B.A. Miller, TRPC2, (2014) 53–65, https://doi.org/10.1007/978-3-642-54215-2_3.
[49] R. de Sousa Abreu, L.O. Penalva, E.M. Marcotte, C. Vogel, Global signatures of protein and mRNA expression levels, Mol. Biosyst. (2009), https://doi.org/10.1039/b908315d.
[50] C. Buccitelli, M. Selbach, mRNAs, proteins and the emerging principles of gene expression control, Nat. Rev. Genet 21 (2020) 630–644, https://doi.org/10.1038/s41576-020-0258-4.
[51] M.J. Kelly, J. Qiu, O.K. Rønnekleiv, TRPCing around the hypothalamus, Front Neuroendocr. 51 (2018) 116–124, https://doi.org/10.1016/j.yfrne.2018.05.004.
[52] M. Onopiuk, B. Eby, V. Nesin, P. Ngo, M. Lerner, C.M. Gorvin, V.J. Stokes, R. V. Thakker, M.L. Brandi, W. Chang, M.B. Humphrey, L. Tsiokas, K. Lau, Control of PTH secretion by the TRPC1 ion channel, JCI Insight 5 (2020), https://doi.org/10.1172/jci.insight.132496.
[53] H. Yamada, M. Yoshida, K. Ito, K. Dezaki, T. Yada, S. Ishikawa, M. Kakei, Potentiation of glucose-stimulated insulin secretion by the GPR40–PLC–TRPC pathway in pancreatic β-cells, Sci. Rep. 6 (2016) 25912, https://doi.org/10.1038/srep25912.
[54] E. Kirstein, D. Schaudien, M. Wagner, C.M. Diebolt, A. Bozzato, T. Tschernig, C. N. Englisch, TRPC3 Is downregulated in primary hyperparathyroidism, Int J. Mol. Sci. 25 (2024) 4392, https://doi.org/10.3390/ijms25084392.
[55] C.N. Englisch, E. Kirstein, C.M. Diebolt, M. Wagner, T. Tschernig, Distribution of TRPC3 and TRPC6 in the human exocrine and endocrine pancreas, Pathol. Res Pr. 260 (2024) 155403, https://doi.org/10.1016/j.prp.2024.155403.
[56] P.J. McMillan, W.M. Hooker, L.J. Deftos, Distribution of calcitonin-containing cells in the human thyroid, Am. J. Anat. 140 (1974) 73–79, https://doi.org/10.1002/aja.1001400106.
[57] H.J. Wolfe, E.F. Voelkel, A.H. Tashjian, Distribution of calcitonin-containing cells in the normal adult human thyroid gland: a correlation of morphology with peptide content, J. Clin. Endocrinol. Metab. 38 (1974) 688–694, https://doi.org/10.1210/jcem-38-4-688, olfe,.
[58] K. O'toole, C. Fenoglio-Preiser, N. Pushparaj, Endocrine changes associated with the human aging process: III. Effect of age on the number of calcitonin immunoreactive cells in the thyroid gland, Hum. Pathol. 16 (1985) 991–1000, https://doi.org/10.1016/S0046-8177(85)80276-8.
[59] A.L. Maia, Siqueira Ferreira, Ceolin, Advanced medullary thyroid cancer: pathophysiology and management, Cancer Manag Res (2013) 57, https://doi.org/10.2147/CMAR.S33105.
[60] T. Jaber, R. Dadu, M.I. Hu, Medullary thyroid carcinoma, Curr. Opin. Endocrinol. Diabetes Obes. 28 (2021) 540–546, https://doi.org/10.1097/MED.0000000000000662.
[61] S. Magaki, S.A. Hojat, B. Wei, A. So, W.H. Yong, Introd. Perform. Immunohistochem. (2019) 289–298, https://doi.org/10.1007/978-1-4939-8935-5_25.
[62] D. HAWES, S.-R. SHI, D.J. DABBS, C.R. TAYLOR, R.J. COTE, Immunohistochemistry. in: Modern Surgical Pathology, Elsevier, 2009, pp. 48–70, https://doi.org/10.1016/B978-1-4160-3966-2.00016-3.
[63] A. Zissler, W. Stoiber, P. Steinbacher, J. Geissenberger, F.C. Monticelli, S. Pittner, Postmortem protein degradation as a tool to estimate the pmi: a systematic review, Diagnostics 10 (2020) 1014, https://doi.org/10.3390/diagnostics10121014.
[64] J. Hayman, M. Oxenham, Estimation of the time since death in decomposed bodies found in Australian conditions, Aust. J. Forensic Sci. 49 (2017) 31–44, https://doi.org/10.1080/00450618.2015.1128972.
[65] G. O'Hurley, E. Sjöstedt, A. Rahman, B. Li, C. Kampf, F. Pontén, W.M. Gallagher, C. Lindskog, Garbage in, garbage out: a critical evaluation of strategies used for validation of immunohistochemical biomarkers, Mol. Oncol. 8 (2014) 783–798, https://doi.org/10.1016/j.molonc.2014.03.008.
[66] C. Montero, The antigen-antibody reaction in immunohistochemistry, J. Histochem. Cytochem. 51 (2003) 1–4, https://doi.org/10.1177/002215540305100101.
[67] J.A. Ramos-Vara, Technical aspects of immunohistochemistry, Vet. Pathol. 42 (2005) 405–426, https://doi.org/10.1354/vp.42-4-405.
[68] C.N. Englisch, D. Röhricht, M. Walz, K. Junker, A. Beckmann, C. Meier, F. Paulsen, M. Jung, T. Tschernig, TRPC6 Is found in distinct compartments of the human kidney, Int. J. Transl. Med. 2 (2022) 156–163, https://doi.org/10.3390/ijtm2020013.
[69] H.R. Harach, K.O. Franssila, Thyroglobulin immunostaining in follicular thyroid carcinoma: relationship to the degree of differentiation and cell type, Histopathology 13 (1988) 43–54, https://doi.org/10.1111/j.1365-2559.1988.tb02002.x.
[70] H.I. Gözii, D. Yavuzer, H. Kaya, S. Vural, H. Sargın, C. Gezen, M. Sargın, S. Akalın, Alterations of NIS expression in functioning thyroid nodules, Kulak Burun Bogaz Ihtis. Derg. 20 (2010) 285–292.

3.3 Publikation 3: “Distribution of TRPC3 and TRPC6 in the human exocrine and endocrine pancreas”

Pathology - Research and Practice 260 (2024) 155403

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Distribution of TRPC3 and TRPC6 in the human exocrine and endocrine pancreas

Colya N. Englisch [a,*], Emilie Kirstein [a], Coline M. Diebolt [a], Mathias Wagner [b], Thomas Tschernig [a]

[a] Institute for Anatomy and Cell Biology, Saarland University, 66421 Homburg, Saarland, Germany
[b] Department of General and Special Pathology, Saarland University Medical Center, 66421 Homburg, Saarland, Germany

ARTICLE INFO

Keywords:
TRPC3
TRPC6
Human
Pancreas
Beta cell
Acinar cell
Ductal cell
Immunohistochemistry

ABSTRACT

Background: Expression and function of TRPC3 and TRPC6 in the pancreas is a controversial topic. Investigation in human tissue is seldom. We aimed to provide here a detailed description of the distribution of TRPC3 and TRPC6 in the human exocrine and endocrine pancreas.
Methods: We collected healthy samples from cadavers ($n = 4$) and visceral surgery ($n = 4$) to investigate the respective expression profiles using immunohistochemical tracing with knockout-validated antibodies.
Results: TRPC3- and TRPC6-proteins were detected in different pancreatic structures including acinar cells, as well as epithelial ductal cells from intercalate, intralobular, and interlobular ducts. Respective connective tissue layers appeared unstained. Endocrine islets of Langerhans were clearly and homogenously immunolabeled by the anti-TRPC3 and anti-TRPC6 antibodies. Insular α, β, γ, and δ cells were conclusively stained, although no secure differentiation of cell types was performed.
Conclusions: Due to aforementioned antibody specificity verification, protein expression in the immunolabeled localizations can be accepted. Our study in human tissue supports previous investigations especially with respect to acinar and insular α and β cells, while other localizations are here reported for the first time to express TRPC3 and TRPC6, ultimately warranting further research.

1. Introduction

The pancreas is an exocrine and endocrine gland of the gastrointestinal tract that is critically involved in digestion and blood sugar homeostasis [30]. The exocrine system relies on the acinar backbone that comprises approximatively 80 % of the total pancreatic volume [4]. The corresponding serous acinar cells produce the pancreatic juice – an enzymatic cocktail containing representatives of the α-amylase, lipase, and protease (e.g., trypsinogen and chymotrypsinogen) families [30]. Human adult specimens physiologically produce one to two liters of pancreatic juice daily [30]. Importantly, the ductal cells modify the pancreatic juice by abundant secretion of sodium bicarbonate (~140 mmol HCO_3^-/liter) [28] to buffer the strong acidity of the imported gastric juice [30]. In contrary, the endocrine pancreas is widespread in form of so-called islets of Langerhans throughout the exocrine background. Each islet of Langerhans is built up of several different endocrine cell types including α, β, γ, and δ cells, that respectively secrete glucagon – a catabolic blood sugar-increasing hormone, insulin – its anabolic blood sugar-reducing pendant, pancreatic polypeptide, and somatostatin (Fig. 1) [30]. Meticulously regulated intracellular calcium concentration oscillations have been suggested to be mandatory for both exocrine and endocrine function with respect to acinar and β cells [21,36]. Store-operated channels (SOC), that are activated by increased cytosolic calcium levels subsequent to inositol 1,4,5-triphosphate (IP_3)-triggered endoplasmic reticulum calcium release, prolong the signaling response of initially receptor-stimulated calcium increase thus enabling various cellular functions including sustained exocytosis [21,35]. In this context, Kim *et al.* found out that TRPC3 is critically involved as SOC in calcium entry responsible for secretory function in acinar cells [21]. TRPC3 belongs to a subset of tetrameric non-selective cation channels referred to as transient receptor potential canonical (TRPC) channels – a subfamily of the transient receptor potential (TRP) family that accounts next to the canonical variation, also so-called melastatin, vanilloid, polycystin, mucolipin, no mechanoreceptor potential C, and

* Correspondence to: Institute for Anatomy and Cell Biology, Saarland University, Building 61, Kirrberger Strasse, 66421 Homburg, Saarland, Germany.
E-mail address: colya.englisch@uni-saarland.de (C.N. Englisch).

https://doi.org/10.1016/j.prp.2024.155403
Received 7 May 2024; Received in revised form 3 June 2024; Accepted 9 June 2024
Available online 10 June 2024

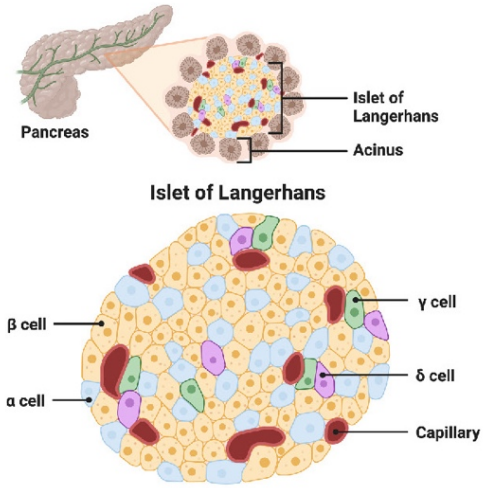


Fig. 1. The endocrine pancreas or the islet of Langerhans. About 80 % of insular cells are insulin-producing β cells. They are rather gathered at the islet's centre, while glucagon-secreting α cells are rather described to accumulate at the islets border. γ (pancreatic polypeptide) and δ (somatostatin) cells are also displayed. Created with BioRender.com (Agreement number: BF25ZPS83F; Toronto, Canada).

ankyrin subfamilies [34]. TRPC channels are expressed in different human tissues where they are suggested to be involved in a wide range of physiological functions (e.g., hearing [12], renal tubular calcium reabsorption [10]) and pathophysiological conditions (e.g., oncogenesis [10], cardiac hypertrophy [9]). As a matter of fact, their suggested involvement also extends to endocrine organs other than the pancreas such as the thyroid [3] or the parathyroid [23] gland for instance. Due to extended sequence homologies [34], TRPC6 – an interesting close subfamily member of TRPC3, has been reported to heteropolymerise with TRPC3 to ultimately increase the functional diversity of TRPC channels [24,48]. Especially TRPC3 has recently been suggested to be involved in insulin secretion in pancreatic β cells [36]. However, contradictory molecular investigations didn't support TRPC3-expression in β cells [32, 37], ultimately foregrounding the need to further investigate TRPC3 in the endocrine pancreas, as indicated by Rached *et al.* [36]. Another relevant aspect represents the aforementioned human exocrine pancreas, which has not been entirely submitted to TRPC channel-focused research yet, although a few studies investigating its serous acini appeared in the past.

To this end, and essentially inspired by Rached *et al.* [36] we investigated here in detail the expression of the non-selective cation channels TRPC3 and TRPC6 in rarely available healthy human pancreatic tissue. Finally, we support TRPC3- and TRPC6-protein expression in previously suggested localizations such as α, β, and acinar cells. Additionally, we provide clear evidence for the expression of these channels in different ductal epithelia, and also suggest their localization in γ and δ cells.

2. Materials and methods

2.1. Samples

Pancreas tissue was either obtained from human adults that decided to donate their bodies to education and science after death ($n = 4$) or from adult patients that had undergone visceral surgery ($n = 4$). All investigations were conducted in an anonymous manner, approved by the Ethics Committee of the Saarland Medical Association (163/20, 130/21 respectively), and performed in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from all involved patients. Body donors had been fixed within 72 hours post-mortem by injection of nitrite pickling salt-ethanol polyethylene glycol fixation (NEP) through the femoral artery [26]. The corresponding pancreatic samples had been removed by punch biopsy during dissection courses at the anatomical institute. Half of the body donors were female, half male. The mean age was 79 with a standard deviation of 3.5 years. The samples obtained by surgery had been fixed within the surgical schedule using 4 % buffered formalin. These pancreatic samples had been extracted in the context of three Whipple pancreaticoduodenectomies and one Traverso-longmire pylorus-preserving pancreaticoduodenectomy. Three of the patients were female, one male. The mean age was 68.5 with a standard deviation of 1.1 year at the surgery time-point. The patients were respectively operated because of an adenocarcinoma of ductal type, a neuroendocrine pancreas tumor, an adenocarcinoma of the distal choledochal duct, and a sessile tubular duodenal adenoma. The here considered samples were tumor free and age-appropriate, as evaluated and labeled by trained pathologists.

2.2. Tissue treatment and histology

Fixed samples were embedded in paraffin, sectioned at a thickness of 4 μm, and mounted on glass slides. Hematoxylin & Eosin (H&E)-stained sections were generated using routine techniques [5]. Briefly, samples were serially rehydrated by incubation in 100 % xylol (15 min; VWR International, Fontenay-sous-Bois, France) followed by decreasingly concentrated ethanol solutions (100 % [10 min], 90 % [5 min], 80 % [5 min]; Central Chemical Storage, Saarland University, Saarbrucken, Germany), stained in Ehrlich hematoxylin (8 min; Carl Roth GmbH & Co, KG, Karlsruhe, Germany), washed in distillated water and blued in fluent water (12 min). Staining in 0.1 % eosin (210 s; Central Chemical Storage, Saarland University, Saarbrucken, Germany) was adjusted in 90 % isopropanol, followed by final serial dehydration using 100 % isopropanol (10 min) and 100 % xylol (15 min). For immunohistochemical staining of TRPC3 and TRPC6 paraffin was removed, and antigen recovery was performed using citrate buffer (60 min, 95 °C). The primary antibody (polyclonal anti-TRPC3, lyophilized, ACC-016, 1:50, Alomone Labs, Jerusalem BioPark, Israel; polyclonal anti-TRPC6, lyophilized, ACC-017, 1:50, Alomone Labs, Jerusalem BioPark, Israel) was applied overnight and at room temperature. Instead of the primary antibody, 1:500 diluted rabbit serum that was kindly donated by Dr. Martin Jung was used for negative controls. A peroxidase labeled secondary antibody (HRP, Horseradish Peroxidase, anti-rabbit goat, A10547; Invitrogen AG, Carlsbad, CA, USA) and diaminobenzidine (DAB; incubation time = 5 min) tetrahydrochloride as chromogen (SK-4103 Vector Laboratories, Burlingame CA, USA) were added to detect the primary antibody. Nuclear counterstaining with hematoxylin (C. Roth, Karlsruhe, Germany) followed. According to the manufacturer's (Alomone Labs, Jerusalem BioPark, Israel) information, the anti-TRPC3 and anti-TRPC6 antibodies are knockout-validated and designed to detect the corresponding channels in mouse, rat, and human tissue. More specifically the anti-TRPC3 antibody (Peptide HKLSEKLNPSVLRC) detects the amino acid residues 822–835 of mouse TRPC3 that is localized to the intracellular COOH (carboxy-)terminus and the anti-TRPC6 antibody (Peptide [C]RRNESQDYLLMDELG) recognizes the amino acid residues 24–38 of the mouse TRPC6 channel that are localized to the intracellular N-terminus of the first transmembrane segment. Detailed description of the molecular structure of these channels is provided elsewhere [43]. Knockout-validation of each of these antibodies was also provided in other previously published reports, making the here presented results trustable [13,15,50].

2.3. Evaluation

Finally, the slides were digitalized using the Nano Zoomer S210

(Hamamatsu, Japan). Microphotographs were taken using the image viewing software NDP.view2 from Hamamatsu (U12388–01, Hamamatsu, Japan). No digital image editing applied.

3. Results

The cadaveric samples we first investigated featured advanced autolysis impeding islet of Langerhans identification. Therefore, healthy samples from visceral surgery were apprehended. The H&E staining (Fig. 2) displayed peripancreatic fat tissue, hemorrhages (following surgery), a lobar archi- and lobular parenchymatous subarchitecture (not shown). Higher magnification allows clear recognition of the parenchymatous structure involving serous acini (Fig. 2A), their draining "intercalate" ducts (Fig. 2B and C), as well as larger intralobular (Fig. 2C, D, and E), and interlobular ducts (Fig. 2F). The major pancreatic duct was unfortunately not displayed. Islets of Langerhans, in turn, are well recognizable (Fig. 2G and H). Nevertheless, tissue quality was different among the samples. We show here microphotographs (Figs. 2,3, and 4) from the sample with less advanced autolysis – if at all – that was surgically obtained due to a sessile tubular duodenal adenoma. The following description includes all samples, unless differently mentioned.

Anti-TRPC3 immunohistochemical staining was detected in exocrine acini (Fig. 3A and B) and in corresponding intercalate ducts (Fig. 3C). In most cases, the entire acinar cells (plasma membrane and cytoplasm) of the section plane were labeled by the chromogen, although intracellular staining intensity was variable. Certain acinar cells featured indeed stronger intracellular staining loci mimicking cell organelles (Fig. 3B).

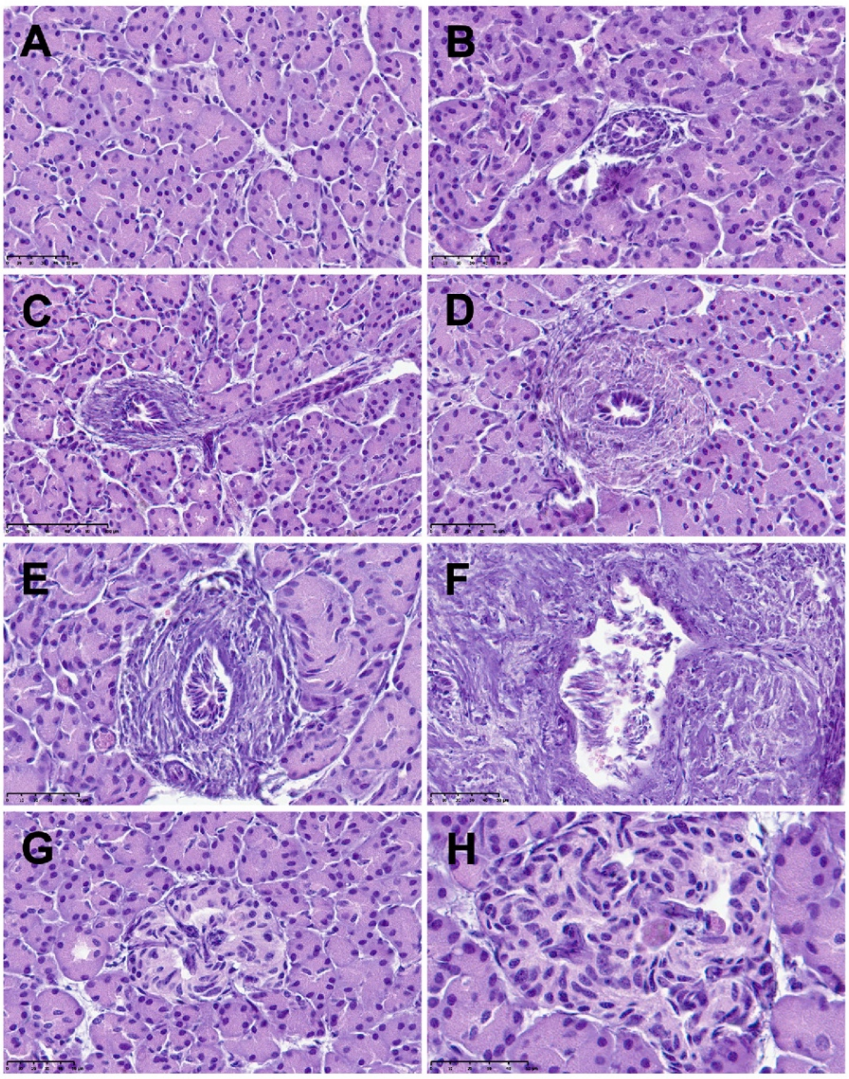


Fig. 2. Hematoxylin & Eosin staining of the pancreas. Exocrine acinous tissue (A). Intercalate duct surrounded by acini (B). An intercalate duct flows into an intralobular analogue (C). Intralobular duct surrounded by acini (D, E). Interlobular duct (F). Islet of Langerhans surrounded by acini (G). Islet of Langerhans surrounded by acini (higher magnification; H).

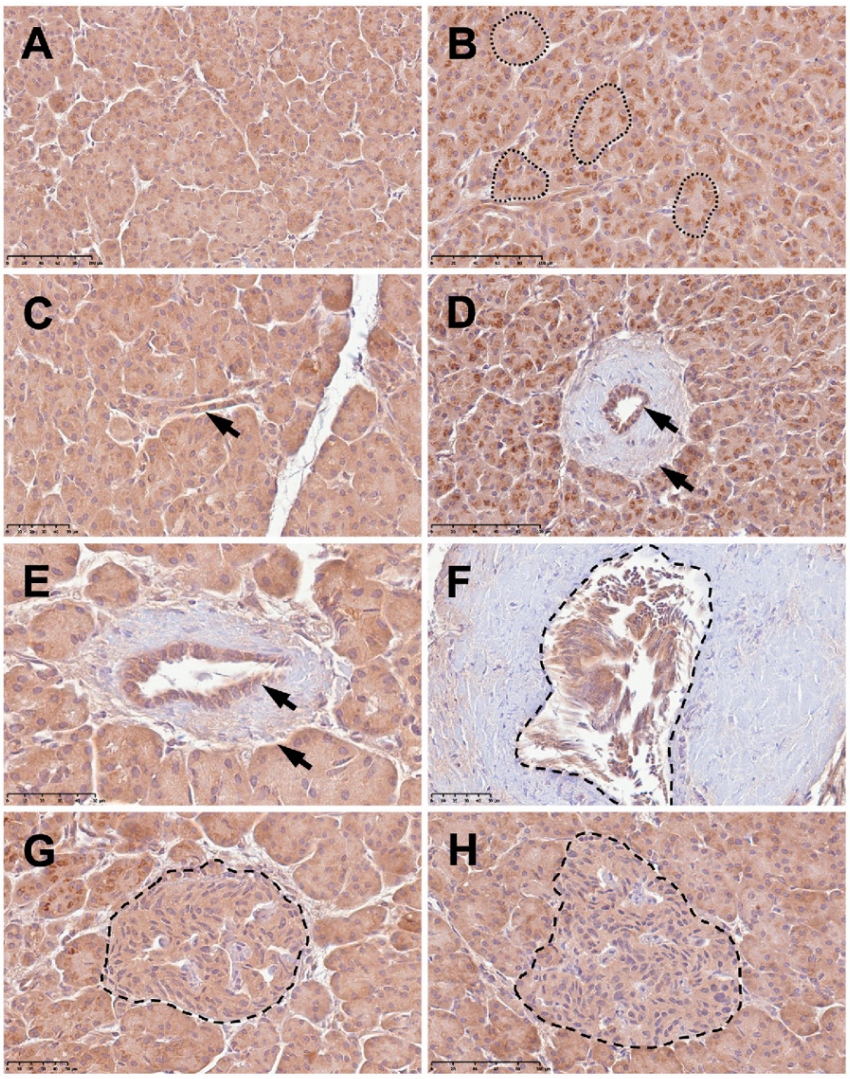


Fig. 3. TRPC3-protein expression in the human pancreas. Pyramid-shaped serous acinar cells (A). Pyramid-shaped serous acinar cells with increased isolated intracellular staining (B). Intercalate duct (arrow) surrounded by acini. Two different lobules are distinguishable (C). Intralobular duct surrounded by acinous tissue as observed in microphotograph B. Inner arrow indicates the ductal epithelium. Outer arrow represents the connective tissue layer (D, E). Interlobular duct. Ductal epithelium is recognizable inside the demarcation, and connective tissue layer outside (F). Islets of Langerhans in lower (G) and higher (H) magnification.

The respective appearances were largely neighbored. However, this staining pattern was essentially observed in the here depicted sample, which may be attributable to varying autolysis in the three remaining samples, that was howsoever noticeably less than in cadaveric tissue (not shown). Intralobular ducts feature highly prismatic epithelial cells that were immunoreactive suggesting TRPC3-protein expression (Fig. 3D and E). In contrast, the surrounding connective tissue layer was immunolabeling-deficient (Fig. 3D and E). Interlobular ducts were seldom, but similarly to intralobular analogues stained with respect to the luminal epithelium (Fig. 3F). The islets of Langerhans were immunoreactive and featured a homogenic signal distribution, thus not indicating clear contrasts among the insular cells with respect to TRPC3-expression. Insular capillaries were distinguished but remained unstained (Fig. 3G and H). Negative controls did not display any DAB-specific brown coloration (not shown).

As a matter of fact, a similar staining distribution was observed when using the anti-TRPC6 antibody (Fig. 4). Indeed, exocrine acini were stained. Again, in most cases the entire acinar cells (plasma membrane and cytoplasm) of the section plane were labeled by the chromogen (Fig. 4A and B). Interestingly, the anti-TRPC3-staining pattern mimicking cell organelles, was not observed with anti-TRPC6-staining in the same sample. The cuboidal cells of the intercalate ducts appeared clearly stained (Fig. 4C). Highly prismatic epithelia of intralobular (Fig. 4D and E) and interlobular (Fig. 4F) ducts were similarly stained, whereas their connective tissue layers were mostly lacking immunolabeling. The endocrine islets of Langerhans were homogenously stained

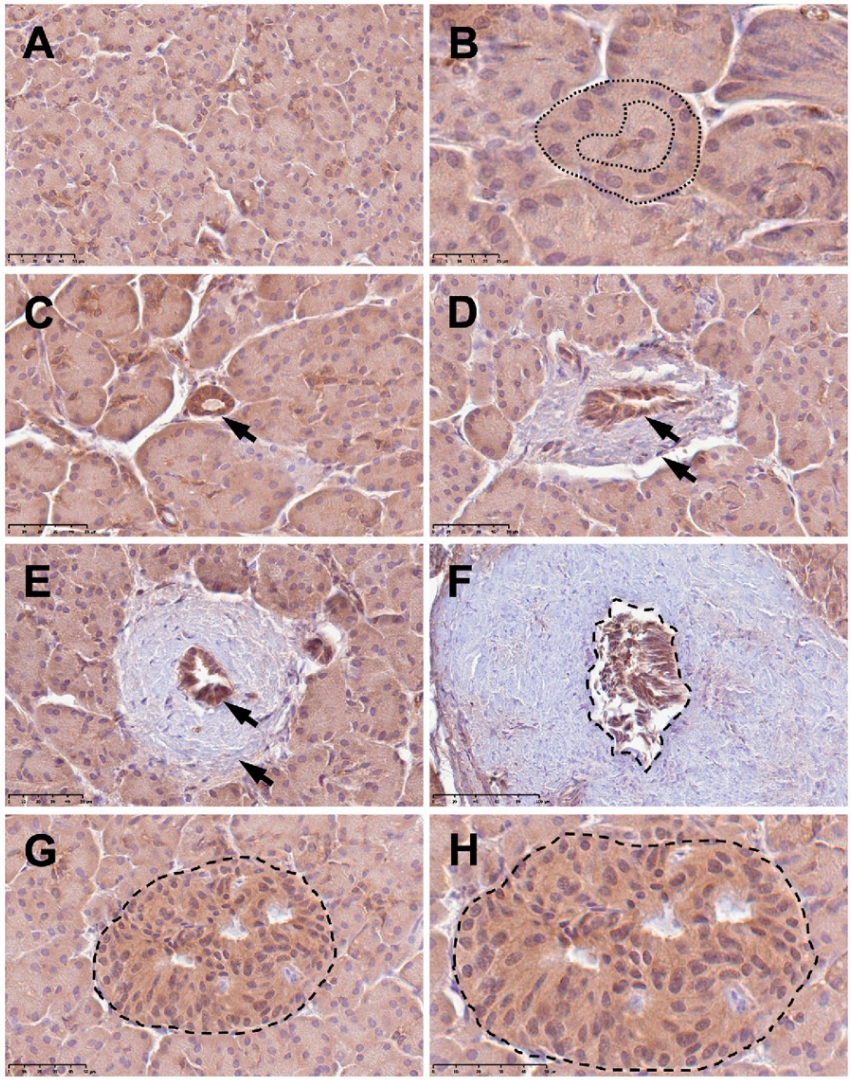


Fig. 4. TRPC6-protein expression in the human pancreas. Pyramid-shaped serous acinar cells in lower (A) and higher magnification (B). The demarcations delimit the acinar cells in the plane. Centroacinar cells are recognizable (B). Intercalate duct (arrow) surrounded by acini (C). Intralobular duct (oblique sectioned plane: D; transversal sectioned plane: E) surrounded by acinous tissue. Inner arrow indicates the ductal epithelium. Outer arrow represents the connective tissue layer (D, E). Interlobular duct. Ductal epithelium is recognizable inside the demarcation, and connective tissue layer outside (F). Islets of Langerhans in lower (G) and higher (H) magnification.

and seemed to tower above the exocrine backbone with respect to staining intensity. Again, unstained insular capillaries were detected (Fig. 4G and H). Negative controls did not display any DAB-specific brown coloration (not shown).

4. Discussion

We provide here evidence that suggests TRPC3- and TRPC6-expression in both the human exocrine and endocrine pancreas. Immunohistochemical protein detection is highly sensitive and can detect small channel amounts. Besides, it enables cellular and subcellular localization of the protein in contrary to other detection methods. Importantly, the use of knockout-validated antibodies as in the present study is a critical factor for results trustability. Such immunohistochemical investigations on the human pancreas, as performed here, are rare, since commonly available pancreatic tissue from cadavers is often impaired in quality. Manifestations such as tissue liquefaction, protein degradation, and other artefacts are known to occur [17,52]. Instead, samples need to be gained from visceral surgery, as previously done [36], which is concomitant with further discussions concerning the integrity and healthiness of the considered tissue. As mentioned above the tissue was quality-checked by trained pathologists and labeled as physiological and age-appropriate. Of course, pancreas tissue from older patients cannot reflect conditions in younger specimens, which restricts

the generalizability of any conclusions. Further, tumors, especially malignant entities can affect protein expression in neighbored healthy tissue. However, since not all of our samples were obtained from cancer-affected organs, and similar patterns were observed independently of the surgery indication, we assumed a good translationability of our results. Also, similar approaches have been chosen previously to investigate protein expression not only in pancreatic islets of Langerhans [36], but also in other organs such as the kidneys for instance [7,11,25]. When interpretating the findings, it has to be kept in mind that our small case number can lead to missing detection of potential variances in the pancreatic protein expression profile. Thus, further studies involving higher case numbers will be needed to verify the here presented results.

We detected TRPC3- and TRPC6-immunostaining throughout all pancreatic samples. The exocrine tissue was widely stained, although certain lobules or lobular segments of the best-preserved sample tended to display noticeable intracellular signals following anti-TRPC3-staining. Indeed, staining of both proteins was in most cases detected in different cellular sublocalizations that included the plasma membrane and the cytoplasm. As a matter of fact, conventional light microscopy does not allow differentiation of cell organelles other than the nucleus [19]. It is however conceivable, that organelles responsible for membrane proteins (e.g., TRPC3 and TRPC6) synthetization, sorting, and trafficking such as the endoplasmic reticulum and the Golgi apparatus conditioned the observed cytoplasmic immunoreaction [39]. Kokubun *et al.* discussed similar staining sources after having described TRPV1 and 2 channel-associated immunoreactivity that was rather localized to the cytoplasm than to the plasma membrane [27]. In addition, further studies reported the cytoplasmic localization of TRP channels in association with their functional mode [1,14,41]. That the anti-TRPC3 immunohistochemical signals were accentuated in certain lobules or lobular segments could be ascribed to differing oxygen supplies that ultimately dictate cellular metabolism [44]. Hepatocytes, for instance, are known to display an alterable metabolism and thus a protein expression depending on oxygen supply [49]. Why this phenomenon was only observed with respect to TRPC3- and not TRPC6-staining is unclear, although TRPC6-distribution was previously suggested not to be affected by altered oxygen supply in the liver [47]. This pattern was essentially observed in the best-preserved sample, which can be ascribed to in relation advanced autolysis in the other samples that renders such cellular or subcellular considerations mechanistically impossible. Of course, fixation artefacts as reason for differing representations cannot be excluded either. Evidently, this rather mimics a case-report, and further studies will be needed to address verification of these findings.

The relevance of these channels in acinar cells was established by Kim *et al.* who first revealed acinar expression of the TRPC3- and TRPC6-proteins in the murine pancreas [20]. Later, they demonstrated TRPC3 to be relevant for physiological exocytosis of pancreatic digestive enzymes by regulating the frequency of physiological cytosolic calcium level oscillations [21]. TRPC3-deletion, that was employed in this study, also reduced stimulated-pathological sustained cytosolic calcium levels, decreased intracellular trypsin activation, and subsequently pancreatitis severity [21]. Beyond, tools including TRPC3-knockout and pharmacological inhibition (TRPC3-inhibitor pyrazole 3 [Pyr3]) were later used in experimentally-induced acute pancreatitis, to suggest that TRPC3 is a critical influx-player in calcium-mediated toxicity and that its inhibition can attenuate the SOC-associated pancreatitis [22]. The study from Du *et al.* supported the relevance of TRPC3 and TRPC6 in acute pancreatitis, by showing that microRNA-26a (miR-26a) targets the SOCs TRPC3 and TRPC6, thus alleviating aforementioned physiological calcium oscillations and pathological sustained calcium elevations in acinar cells [8]. On one hand, miR-26a-deletion, led to increased SOC-expression, ultimately exacerbating acute pancreatitis, while on the other hand miR-26a-upregulation remarkably attenuated the histopathological manifestations [8]. Together with our detailed histological studies in human pancreas tissue, there is clear morphological and experimental evidence for the benefit of TRPC3- and possibly TRPC6-inhibition as therapeutic tool in patients suffering from pancreatitis.

Investigations of pancreatic duct cells with respect to TRPC3- or TRPC6-expression are significantly less compared to acinar cell studies, and probably even not existent to the authors knowledge. Since, we detected immunohistochemical signals in diverse pancreatic ducts, questions arise concerning their function in these localizations. In tissue analogy, functional TRPC3 was detected in salivary ductal cells [6]. Again, morphological and experimental studies will be needed to verify our results by higher case numbers and to explore the role of TRPC3 but also of TRPC6 in these eagerly bicarbonate-secreting cells. Besides the aforementioned exocrine, endocrine, and ductal cells, pancreatic stellate cells represent approximatively 7 % of all pancreatic cells, and have been shown to be implied in pancreas pathologies [18]. These stellate cells are commonly detected using specific immunohistochemical targets (e.g., vimentin, desmin, neural growth factor) [2,18], which has not been performed here. However, there are studies that suggest relevance of TRPC3 and TRPC6 in these cells. For instance, TRPC6 was shown to modulate the hypoxia-response [33], while TRPC3 was recognized to be relevant for cell migration especially promoting tissue fibrosis and thus disease progression in the context of pancreatic ductal adenocarcinoma, in which the channel was upregulated [31,40].

As mentioned above TRPC3-expression is less than clear in the endocrine pancreas. While certain studies didn't identify TRPC3 in human pancreatic β cells [32,37], others observed that TRPC3-blockade neither altered basal nor stimulated-insulin secretion in rat insulinoma cell lines INS-1E [38]. In the same time period, however, both TRPC3 and TRPC6 were proven to be involved in α and β cell proliferation [16], while only TRPC3 was shown to be implicated in PLC (phospholipase C)/PKC (protein kinase C)/GPR40 (G protein-coupled receptor 40) pathways resulting in cytosolic $[Ca^{2+}]$-elevation leading to insulin secretion in rat β cells [51]. Recently, Rached *et al.* demonstrated through immunofluorescence colocalization of TRPC3 and insulin in human pancreatic islets, ultimately suggesting TRPC3-expression in human β cells [36]. Similar patterns were observed in mice. Since anti-TRPC3-signature was also detected at the islet's borders, TRPC3-expression was indicated in human α cells, as supported by our study [36]. Functional relevance in these cells was also conceivable, since glucagon secretion is similarly calcium-dependent, although differently regulated than insulin secretion in β cells [45]. In this context, Takatani *et al.* provided evidence supporting the involvement of *trpc3* gene in insulin-induced and IRS1 (insulin receptor substrate 1)-mediated glucagon regulation in α cells [42]. Morphological aspects such as the respective islet localization of α and β cells and the centrifugal insular blood flow play here an essential role [29,30]. Beside presenting morphological findings, that are deepened by our results, Rached *et al.* further identified TRPC3 to be essentially involved in glucose-stimulated insulin secretion [36], which contrasts the aforementioned results from Sabourin *et al.* [38]. Rached *et al.* [36] tried to explain these discrepancies by use of suboptimal pharmacological inhibition and cell line in the reported publication [38]. Altogether, these experimental studies highlight the relevance of TRPC3 and/or TRPC6 in the endocrine system as well as its interest as therapeutic target. However, some of these studies didn't refer to protein detection, and only few were based on human tissue. From this point of view, our study in human tissue supports most of the corresponding studies suggesting TRPC3- and TRPC6-expression in islets of Langerhans. As a matter of fact, no relevant staining differences were observed among the insular cells in our study, suggesting a wide expression quite certainly involving α and β cells due to their high insular proportion and possibly also γ and δ cells, in which these cation channels haven't been investigated yet to our best knowledge.

In conclusion, we verified here the wide expression pattern of TRPC3 and TRPC6 in the human pancreas, after their relevance was suggested in different compartments including exocrine acinar, as well as endocrine α and β cells. Distinct localizations that include pancreatic ductal cells, as well as possibly endocrine γ and δ cells warrant experimental

research with respect to TRPC channel function.

Ultimately our study supports the concept of therapeutical TRPC3 and/or TRPC6 channel activation/modulation/inhibition [46] in exocrine (e.g., pancreatitis) and endocrine (e.g., diabetes mellitus) pancreatic conditions, that nevertheless needs to be further investigated.

Ethics statement

All investigations were implemented anonymously, approved by the Ethics Committee of the Saarland Medical Association (163/20, 130/21), and performed in accordance with the guidelines of the Declaration of Helsinki. Informed consent was obtained from all involved patients.

Funding

None.

CRediT authorship contribution statement

Thomas Tschernig: Writing – review & editing, Supervision, Resources, Project administration, Methodology, Conceptualization. **Mathias Wagner:** Writing – review & editing, Resources. **Coline Diebolt:** Writing – review & editing, Methodology. **Emilie Kirstein:** Writing – review & editing, Methodology, Investigation. **Colya Englisch:** Writing – original draft, Visualization, Supervision, Conceptualization.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank Dirk Schaudien for technical and Martin Jung, Anja Beckmann, as well as Carola Meier for material support. Our gratitude goes to those who donated their bodies to science so that anatomical research could be performed. Results from such research can potentially increase mankind's overall knowledge, eventually improving patient care. Therefore, these donors and their families deserve our highest gratitude.

References

[1] L. Albarran, N. Dionisio, E. Lopez, G.M. Salido, P.C. Redondo, J.A. Rosado, STIM1 regulates TRPC6 heteromultimerization and subcellular location, Biochem. J. 463 (2014) 373–381.
[2] M.V. Apte, P.S. Haber, T.L. Applegate, I.D. Norton, G.W. McCaughan, M.A. Korsten, R.C. Pirola, J.S. Wilson, Periacinar stellate shaped cells in rat pancreas: identification, isolation, and culture, Gut 43 (1998) 128–133.
[3] M.Y. Asghar, M. Magnusson, K. Kemppainen, P. Sukumaran, C. Lof, I. Pulli, V. Kalhori, K. Tornquist, Transient Receptor Potential Canonical 1 (TRPC1) channels as regulators of sphingolipid and VEGF receptor expression: implications for thyroid cancer cell migration and proliferation, J. Biol. Chem. 290 (2015) 16116–16131.
[4] R.P. Bolender, Stereological analysis of the guinea pig pancreas. I. Analytical model and quantitative description of nonstimulated pancreatic exocrine cells, J. Cell Biol. 61 (1974) 269–287.
[5] R.D. Cardiff, C.H. Miller, R.J. Munn, Manual hematoxylin and eosin staining of mouse tissue sections, Cold Spring Harb. Protoc. (2014) 655–658.
[6] B.E. Choi, S. Shin, S. Evans, B.B. Singh, B.C. Bandyopadhyay, Ablation of TRPC3 disrupts Ca(2+) signaling in salivary ductal cells and promotes sialolithiasis, Sci. Rep. 13 (2023) 5772.
[7] C.M. Diebolt, D. Schaudien, K. Junker, G. Krasteva-Christ, T. Tschernig, C. N. Englisch, New insights in the renal distribution profile of TRPC3 - Of mice and men, Ann. Anat. 252 (2024) 152192.
[8] W. Du, G. Liu, N. Shi, D. Tang, P.E. Ferdek, M.A. Jakubowska, S. Liu, X. Zhu, J. Zhang, L. Yao, X. Sang, S. Zou, T. Liu, R. Mukherjee, D.N. Criddle, X. Zheng, Q. Xia, P.O. Berggren, W. Huang, R. Sutton, Y. Tian, W. Huang, X. Fu, A microRNA checkpoint for Ca(2+) signaling and overload in acute pancreatitis, Mol. Ther. 30 (2022) 1754–1774.
[9] P. Eder, J.D. Molkentin, TRPC channels as effectors of cardiac hypertrophy, Circ. Res. 108 (2011) 265–272.
[10] C.N. Englisch, F. Paulsen, T. Tschernig, TRPC channels in the physiology and pathophysiology of the renal tubular system: what do we know? Int. J. Mol. Sci. 24 (2022).
[11] C.N. Englisch, D. Röhricht, M. Walz, K. Junker, A. Beckmann, C. Meier, F. Paulsen, M. Jung, T. Tschernig, TRPC6 is found in distinct compartments of the human kidney, Int. J. Transl. Med. 2 (2022) 156–163.
[12] C.N. Englisch, J. Steinhauser, S. Wemmert, M. Jung, J. Gawlitza, G. Wenzel, B. Schick, T. Tschernig, Immunohistochemistry reveals TRPC channels in the human hearing organ-A novel CT-guided approach to the Cochlea, Int. J. Mol. Sci. 24 (2023).
[13] S. Feng, H. Li, Y. Tai, J. Huang, Y. Su, J. Abramowitz, M.X. Zhu, L. Birnbaumer, Y. Wang, Canonical transient receptor potential 3 channels regulate mitochondrial calcium uptake, Proc. Natl. Acad. Sci. USA 110 (2013) 11011–11016.
[14] M. Ghavideldarestani, A.E. Butler, S. Shirian, S.L. Atkin, Expression and localization of transient receptor potential channels in the bovine uterus epithelium throughout the estrous cycle, Mol. Biol. Rep. 46 (2019) 4077–4084.
[15] M.T. Harper, J.E. Londono, K. Quick, J.C. Londono, V. Flockerzi, S.E. Philipp, L. Birnbaumer, M. Freichel, A.W. Poole, Transient receptor potential channels function as a coincidence signal detector mediating phosphatidylserine exposure, Sci. Signal 6 (2013) ra50.
[16] H.L. Hayes, L.G. Moss, J.C. Schisler, J.M. Haldeman, Z. Zhang, P.B. Rosenberg, C. B. Newgard, H.E. Hohmeier, Pdx-1 activates islet alpha- and beta-cell proliferation via a mechanism regulated by transient receptor potential cation channels 3 and 6 and extracellular signal-regulated kinases 1 and 2, Mol. Cell Biol. 33 (2013) 4017–4029.
[17] J. Hayman, M. Oxenham, Estimation of the time since death in decomposed bodies found in Australian conditions, Aust. J. Forensic Sci. 49 (2017) 31–44.
[18] P. Hrabak, M. Kalousova, T. Krechler, T. Zima, Pancreatic stellate cells - rising stars in pancreatic pathologies, Physiol. Res. (2021) S597–S616.
[19] S. Jakobs, T. Stephan, P. Ilgen, C. Bruser, Light microscopy of mitochondria at the nanoscale, Annu Rev. Biophys. 49 (2020) 289–308.
[20] J.Y. Kim, W. Zeng, K. Kiselyov, J.P. Yuan, M.H. Dehoff, K. Mikoshiba, P.F. Worley, S. Muallem, Homer 1 mediates store- and inositol 1,4,5-trisphosphate receptor-dependent translocation and retrieval of TRPC3 to the plasma membrane, J. Biol. Chem. 281 (2006) 32540–32549.
[21] M.S. Kim, J.H. Hong, Q. Li, D.M. Shin, J. Abramowitz, L. Birnbaumer, S. Muallem, Deletion of TRPC3 in mice reduces store-operated Ca2+ influx and the severity of acute pancreatitis. Gastroenterology 137 (2009) 1509–1517.
[22] M.S. Kim, K.P. Lee, D. Yang, D.M. Shin, J. Abramowitz, S. Kiyonaka, L. Birnbaumer, Y. Mori, S. Muallem, Genetic and pharmacologic inhibition of the Ca2+ influx channel TRPC3 protects secretory epithelia from Ca2+-dependent toxicity, Gastroenterology 140 (2011), 2107-2115, 2115 e2101-2104.
[23] E. Kirstein, D. Schaudien, M. Wagner, C.M. Diebolt, A. Bozzato, T. Tschernig, C. N. Englisch, TRPC3 Is downregulated in primary hyperparathyroidism, Int. J. Mol. Sci. 25 (2024).
[24] K. Kiselyov, R.L. Patterson, The integrative function of TRPC channels, Front. Biosci. 14 (2009) 45–58.
[25] A.D. Kistler, G. Singh, M.M. Altintas, H. Yu, I.C. Fernandez, C. Gu, C. Wilson, S. K. Srivastava, A. Dietrich, K. Walz, D. Kerjaschki, P. Ruiz, S. Dryer, S. Sever, A. K. Dinda, C. Faul, J. Reiser, Transient receptor potential channel 6 (TRPC6) protects podocytes during complement-mediated glomerular disease, J. Biol. Chem. 288 (2013) 36598–36609.
[26] R. Klopfleisch, M. von Deetzen, A.T. Weiss, J. Weigner, F. Weigner, J. Plendl, A. D. Gruber, Weigners fixative-an alternative to formalin fixation for histology with improved preservation of nucleic acids, Vet. Pathol. 50 (2013) 191–199.
[27] S. Kokubun, T. Sato, C. Ogawa, K. Kudo, K. Goto, Y. Fujii, Y. Shimizu, H. Ichikawa, Distribution of TRPV1 and TRPV2 in the human stellate ganglion and spinal cord, Neurosci. Lett. 590 (2015) 6–11.
[28] M.G. Lee, E. Ohana, H.W. Park, D. Yang, S. Muallem, Molecular mechanism of pancreatic and salivary gland fluid and HCO3 secretion, Physiol. Rev. 92 (2012) 39–74.
[29] P.S. Leung, Overview of the pancreas, Adv. Exp. Med. Biol. 690 (2010) 3–12.
[30] P.S. Leung, Physiology of the pancreas, Adv. Exp. Med. Biol. 690 (2010) 13–27.
[31] Y. Liu, Y. Lyu, H. Wang, TRP channels as molecular targets to relieve endocrine-related diseases, Front. Mol. Biosci. 9 (2022) 895814.
[32] F. Marabita, M.S. Islam, Expression of transient receptor potential channels in the purified human pancreatic beta-cells, Pancreas 46 (2017) 97–101.
[33] N. Nielsen, K. Kondratska, T. Ruck, B. Hild, I. Kovalenko, S. Schimmelpfennig, J. Welzig, S. Sargin, O. Lindemann, S. Christian, S.G. Meuth, N. Prevarskaya, A. Schwab, TRPC6 channels modulate the response of pancreatic stellate cells to hypoxia, Pflug. Arch. 469 (2017) 1567–1577.
[34] B. Nilius, G. Owsianik, The transient receptor potential family of ion channels, Genome Biol. 12 (2011) 218.
[35] M. Prakriya, R.S. Lewis, Store-operated calcium channels, Physiol. Rev. 95 (2015) 1383–1436.
[36] G. Rached, Y. Saliba, D. Maddah, J. Hajal, V. Smayra, J.J. Bakhos, K. Groschner, L. Birnbaumer, N. Fares, TRPC3 regulates Islet beta-cell insulin secretion, Adv. Sci. 10 (2023) e2204846.
[37] P. Rorsman, F.M. Ashcroft, Pancreatic beta-cell electrical activity and insulin secretion: of mice and men, Physiol. Rev. 98 (2018) 117–214.
[38] J. Sabourin, L. Le Gal, L. Saurwein, J.A. Haefliger, E. Raddatz, F. Allagnat, Store-operated Ca2+ entry mediated by Orai1 and TRPC1 participates to insulin secretion in rat beta-cells. J. Biol. Chem. 290 (2015) 30530–30539.

[39] J. Smirle, C.E. Au, M. Jain, K. Dejgaard, T. Nilsson, J. Bergeron, Cell biology of the endoplasmic reticulum and the Golgi apparatus through proteomics, Cold Spring Harb. Perspect. Biol. 5 (2013) a015073.
[40] H. Storck, B. Hild, S. Schimmelpfennig, S. Sargin, N. Nielsen, A. Zaccagnino, T. Budde, I. Novak, H. Kalthoff, A. Schwab, Ion channels in control of pancreatic stellate cell migration, Oncotarget 8 (2017) 769–784.
[41] N. Tajeddine, N. Zanou, M. Van Schoor, J. Lebacq, P. Gailly, TRPC1: subcellular localization? J. Biol. Chem. 285 (2010) le1; author reply le2.
[42] T. Takatani, J. Shirakawa, K. Shibue, M.K. Gupta, H. Kim, S. Lu, J. Hu, M.F. White, R.T. Kennedy, R.N. Kulkarni, Insulin receptor substrate 1, but not IRS2, plays a dominant role in regulating pancreatic alpha cell function in mice, J. Biol. Chem. 296 (2021) 100646.
[43] Q. Tang, W. Guo, L. Zheng, J.X. Wu, M. Liu, X. Zhou, X. Zhang, L. Chen, Structure of the receptor-activated human TRPC6 and TRPC3 ion channels, Cell Res. 28 (2018) 746–755.
[44] C.T. Taylor, C.C. Scholz, The effect of HIF on metabolism and immunity, Nat. Rev. Nephrol. 18 (2022) 573–587.
[45] A. Tengholm, E. Gylfe, cAMP signalling in insulin and glucagon secretion, Diabetes Obes. Metab. 19 (Suppl 1) (2017) 42–53.
[46] O. Tiapko, K. Groschner, TRPC3 as a target of novel therapeutic interventions, Cells 7 (2018).
[47] M. Walz, T. Tschernig, P. Schmidt, J.M. Federspiel, TRPC6-protein expression in the elderly and in liver disease, Ann. Anat. 245 (2023) 152016.
[48] H. Wang, X. Cheng, J. Tian, Y. Xiao, T. Tian, F. Xu, X. Hong, M.X. Zhu, TRPC channels: Structure, function, regulation and recent advances in small molecular probes, Pharmacol. Ther. 209 (2020) 107497.
[49] U. Welsch, W. Kummer, T. Deller. Histologie - Das Lehrbuch, [Histology - The Textbook], 5th ed., Urban & Fischer Verlag/Elsevier GmbH, München, 2018.
[50] W. Xia, Q. Wang, S. Lin, Y. Wang, J. Zhang, H. Wang, X. Yang, Y. Hu, H. Liang, Y. Lu, Z. Zhu, D. Liu, A high-salt diet promotes hypertrophic scarring through TRPC3-mediated mitochondrial Ca(2+) homeostasis dysfunction. Heliyon 9 (2023) e18629.
[51] H. Yamada, M. Yoshida, K. Ito, K. Dezaki, T. Yada, S.E. Ishikawa, M. Kakei, Potentiation of glucose-stimulated insulin secretion by the GPR40-PLC-TRPC pathway in pancreatic beta-cells, Sci. Rep. 6 (2016) 25912.
[52] A. Zissler, W. Stoiber, P. Steinbacher, J. Geissenberger, F.C. Monticelli, S. Pittner, Postmortem protein degradation as a tool to estimate the PMI: a systematic review, Diagnostics 10 (2020).

4. Synthese und Diskussion

4.1 Zusammenfassung der wichtigsten Erkenntnisse

Die drei Studien liefern neue Erkenntnisse über die Verteilung und von TRPC-Kanälen in hormonproduzierenden Organen des menschlichen Körpers.

Die Untersuchung der Expression von TRPC3 in der Nebenschilddrüse zeigt, dass dieses Protein bei Patienten mit pHPT signifikant herunterreguliert ist, während TRPC6 in diesem Zusammenhang keine Veränderungen aufweist.[61] Dies deutet darauf hin, dass TRPC3 eine spezifische regulatorische Rolle bei der Sekretion von PTH spielen könnte, was für die Erforschung von Störungen des Ca^{2+}-Stoffwechsels von besonderem Interesse ist.

Die Untersuchung der Expression von TRPC3 und TRPC6 in der menschlichen Schilddrüse zeigt, dass diese Kanäle sowohl in Thyreozyten als auch in C-Zellen exprimiert werden.[60] Die heterogene Expression in Schilddrüsenzellen deutet darauf hin, dass TRPC-Kanäle eine selektive regulatorische Funktion bei der Produktion und Freisetzung von Schilddrüsenhormonen haben könnten. Darüber hinaus könnte ihr Vorkommen in C-Zellen, die für die Calcitonin-Sekretion verantwortlich sind, auf eine Beteiligung an der Ca^{2+}-Homöostase hindeuten.

Die Untersuchung der Expression von TRPC3 und TRPC6 im exokrinen und endokrinen Pankreas zeigt, dass beide Proteine in azinären Zellen, Gangepithelzellen und Langerhans-Inseln exprimiert werden.[30] Dies weist auf eine mögliche Funktion sowohl in der exokrinen Sekretion als auch in der Regulation der Insulinfreisetzung im Menschen hin, die vorab experimentell angedeutet wurde.

4.2 Beitrag zur Forschung über TRPC-Kanäle

Zusammenfassend tragen diese Studien wesentlich zum Verständnis der Verteilung und potenziellen Funktion von TRPC-Kanälen im endokrinen System im Menschen bei. Während die Herunterregulation von TRPC3 in der Nebenschilddrüse eine neue pathophysiologische Perspektive für pHPT eröffnet, unterstreicht die weitreichende Expression von TRPC3 und TRPC6 im Pankreas und in der Schilddrüse ihre potenzielle Rolle in hormonellen Regulationsmechanismen. Diese Erkenntnisse bilden die Grundlage für weiterführende Untersuchungen zur funktionellen Bedeutung von TRPC-Kanälen in der endokrinen Physiologie und möglichen therapeutischen Zielstrukturen für assoziierte endokrine Erkrankungen.

5. Literaturverzeichnis

1. Abramowitz J, Birnbaumer L. Physiology and pathophysiology of canonical transient receptor potential channels. *The FASEB Journal*. 2009;23(2):297-328. doi:10.1096/fj.08-119495
2. Alavi MS, Soheili V, Roohbakhsh A. The role of transient receptor potential (TRP) channels in phagocytosis: A comprehensive review. *Eur J Pharmacol*. 2024;964:176302. doi:10.1016/j.ejphar.2023.176302
3. Allen E, Fingeret A. *Anatomy, Head and Neck, Thyroid*.; 2023.
4. Ambudkar IS, Bandyopadhyay BC, Liu X, Lockwich TP, Paria B, Ong HL. Functional organization of TRPC-Ca2+ channels and regulation of calcium microdomains. *Cell Calcium*. 2006;40(5-6):495-504. doi:10.1016/j.ceca.2006.08.011
5. Armstrong M, Asuka E, Fingeret A. *Physiology, Thyroid Function*.; 2023.
6. Asghar MY, Lassila T, Törnquist K. Calcium Signaling in the Thyroid: Friend and Foe. *Cancers (Basel)*. 2021;13(9):1994. doi:10.3390/cancers13091994
7. Asghar MY, Magnusson M, Kemppainen K, et al. Transient Receptor Potential Canonical 1 (TRPC1) Channels as Regulators of Sphingolipid and VEGF Receptor Expression. *Journal of Biological Chemistry*. 2015;290(26):16116-16131.
8. Asghar MY, Törnquist K. Transient Receptor Potential Canonical (TRPC) Channels as Modulators of Migration and Invasion. *Int J Mol Sci*. 2020;21(5):1739. doi:10.3390/ijms21051739
9. Ashcroft FM, Rorsman P. Diabetes Mellitus and the β Cell: The Last Ten Years. *Cell*. 2012;148(6):1160-1171. doi:10.1016/j.cell.2012.02.010
10. Barclay JW, Morgan A, Burgoyne RD. Calcium-dependent regulation of exocytosis. *Cell Calcium*. 2005;38(3-4):343-353. doi:10.1016/j.ceca.2005.06.012
11. Bianco AC, Kim BW. Deiodinases: implications of the local control of thyroid hormone action. *Journal of Clinical Investigation*. 2006;116(10):2571-2579. doi:10.1172/JCI29812
12. Bootman MD, Bultynck G. Fundamentals of Cellular Calcium Signaling: A Primer. *Cold Spring Harb Perspect Biol*. 2020;12(1):a038802. doi:10.1101/cshperspect.a038802
13. Brent GA. Mechanisms of thyroid hormone action. *Journal of Clinical Investigation*. 2012;122(9):3035-3043. doi:10.1172/JCI60047
14. Brissova M, Powers AC. Revascularization of Transplanted Islets. *Diabetes*. 2008;57(9):2269-2271. doi:10.2337/db08-0814
15. Brown EM. Role of the calcium-sensing receptor in extracellular calcium homeostasis. In: *Best Practice and Research: Clinical Endocrinology and Metabolism*. Vol 27. Bailliere Tindall Ltd; 2013:333-343. doi:10.1016/j.beem.2013.02.006

16. Brown MB, Limaiem F. *Histology, Parathyroid Gland*.; 2024.
17. Chang HH, Cheng YC, Tsai WC, Tsao MJ, Chen Y. Pyr3 Induces Apoptosis and Inhibits Migration in Human Glioblastoma Cells. *Cellular Physiology and Biochemistry*. 2018;48(4):1694-1702. doi:10.1159/000492293
18. Clarson LH, Roberts VHJ, Hamark B, Elliott AC, Powell T. Store-operated Ca^{2+} entry in first trimester and term human placenta. *J Physiol*. 2003;550(2):515-528. doi:10.1113/jphysiol.2003.044149
19. Cole BA, Becker EBE. Modulation and Regulation of Canonical Transient Receptor Potential 3 (TRPC3) Channels. *Cells*. 2023;12(18):2215. doi:10.3390/cells12182215
20. Dang AK, Murtazina DA, Magee C, Navratil AM, Clay CM, Amberg GC. GnRH Evokes Localized Subplasmalemmal Calcium Signaling in Gonadotropes. *Molecular Endocrinology*. 2014;28(12):2049-2059. doi:10.1210/me.2014-1208
21. Diebolt CM, Schaudien D, Junker K, Krasteva-Christ G, Tschernig T, Englisch CN. New insights in the renal distribution profile of TRPC3 – Of mice and men. *Annals of Anatomy - Anatomischer Anzeiger*. 2024;252:152192. doi:10.1016/j.aanat.2023.152192
22. Dietrich A, Gudermann T. TRPC6: Physiological Function and Pathophysiological Relevance. In: ; 2014:157-188. doi:10.1007/978-3-642-54215-2_7
23. Dietrich A, Mederos y Schnitzler M, Gollasch M, et al. Increased Vascular Smooth Muscle Contractility in *TRPC6* $^{-/-}$ Mice. *Mol Cell Biol*. 2005;25(16):6980-6989. doi:10.1128/MCB.25.16.6980-6989.2005
24. Dolenšek J, Rupnik MS, Stožer A. Structural similarities and differences between the human and the mouse pancreas. *Islets*. 2015;7(1):e1024405. doi:10.1080/19382014.2015.1024405
25. Dryer SE, Reiser J. TRPC6 channels and their binding partners in podocytes: role in glomerular filtration and pathophysiology. *American Journal of Physiology-Renal Physiology*. 2010;299(4):F689-F701. doi:10.1152/ajprenal.00298.2010
26. Dryer SE, Roshanravan H, Kim EY. TRPC channels: Regulation, dysregulation and contributions to chronic kidney disease. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2019;1865(6):1041-1066. doi:10.1016/j.bbadis.2019.04.001
27. Du W, Liu G, Shi N, et al. A microRNA checkpoint for Ca2+ signaling and overload in acute pancreatitis. *Molecular Therapy*. 2022;30(4):1754-1774. doi:10.1016/j.ymthe.2022.01.033
28. Englisch CN, Diebolt CM, Kirstein E, et al. TRPM6 in murine kidneys—of targets and antibodies. *Naunyn Schmiedebergs Arch Pharmacol*. Published online March 1, 2025. doi:10.1007/s00210-025-03951-0

29. Englisch CN, Diebolt CM, Schaudien D, et al. Proximal Tubular TRPC3 Immunostaining Is Reduced in Human Nephrocalcinosis. *Laboratory Investigation*. 2024;104(9):102109. doi:10.1016/j.labinv.2024.102109
30. Englisch CN, Kirstein E, Diebolt CM, Wagner M, Tschernig T. Distribution of TRPC3 and TRPC6 in the human exocrine and endocrine pancreas. *Pathol Res Pract*. 2024;260:155403. doi:10.1016/j.prp.2024.155403
31. Englisch CN, Paulsen F, Tschernig T. TRPC Channels in the Physiology and Pathophysiology of the Renal Tubular System: What Do We Know? *Int J Mol Sci*. 2022;24(1):181. doi:10.3390/ijms24010181
32. Englisch CN, Röhricht D, Walz M, et al. TRPC6 Is Found in Distinct Compartments of the Human Kidney. *International Journal of Translational Medicine*. 2022;2(2):156-163. doi:10.3390/ijtm2020013
33. Englisch CN, Steinhäuser J, Wemmert S, et al. Immunohistochemistry Reveals TRPC Channels in the Human Hearing Organ—A Novel CT-Guided Approach to the Cochlea. *Int J Mol Sci*. 2023;24(11):9290. doi:10.3390/ijms24119290
34. Fan C, Choi W, Sun W, Du J, Lü W. Structure of the human lipid-gated cation channel TRPC3. *Elife*. 2018;7. doi:10.7554/eLife.36852
35. Fekete C, Lechan RM. Central Regulation of Hypothalamic-Pituitary-Thyroid Axis Under Physiological and Pathophysiological Conditions. *Endocr Rev*. 2014;35(2):159-194. doi:10.1210/er.2013-1087
36. Goel M, Zuo CD, Sinkins WG, Schilling WP. TRPC3 channels colocalize with Na^+/Ca^{2+} exchanger and Na^+ pump in axial component of transverse-axial tubular system of rat ventricle. *American Journal of Physiology-Heart and Circulatory Physiology*. 2007;292(2):H874-H883. doi:10.1152/ajpheart.00785.2006
37. Gonzalez-Cobos JC. TRPC channels in smooth muscle cells. *Frontiers in Bioscience*. 2010;15(1):1023. doi:10.2741/3660
38. Gustavsson N, Wu B, Han W. Calcium Sensing in Exocytosis. In: ; 2012:731-757. doi:10.1007/978-94-007-2888-2_32
39. Hartmann J, Dragicevic E, Adelsberger H, et al. TRPC3 Channels Are Required for Synaptic Transmission and Motor Coordination. *Neuron*. 2008;59(3):392-398. doi:10.1016/j.neuron.2008.06.009
40. Hartmann J, Henning HA, Konnerth A. mGluR1/TRPC3-mediated Synaptic Transmission and Calcium Signaling in Mammalian Central Neurons. *Cold Spring Harb Perspect Biol*. 2011;3(4):a006726-a006726. doi:10.1101/cshperspect.a006726
41. Hartmann J, Konnerth A. TRPC3-dependent synaptic transmission in central mammalian neurons. *J Mol Med*. 2015;93(9):983-989. doi:10.1007/s00109-015-1298-7

42. Hayes HL, Moss LG, Schisler JC, et al. Pdx-1 Activates Islet α- and β-Cell Proliferation via a Mechanism Regulated by Transient Receptor Potential Cation Channels 3 and 6 and Extracellular Signal-Regulated Kinases 1 and 2. *Mol Cell Biol*. 2013;33(20):4017-4029. doi:10.1128/MCB.00469-13
43. Henry BM, Skinningsrud B, Saganiak K, Pękala PA, Walocha JA, Tomaszewski KA. Development of the human pancreas and its vasculature — An integrated review covering anatomical, embryological, histological, and molecular aspects. *Annals of Anatomy - Anatomischer Anzeiger*. 2019;221:115-124. doi:10.1016/j.aanat.2018.09.008
44. Hofmann T, Schaefer M, Schultz G, Gudermann T. Subunit composition of mammalian transient receptor potential channels in living cells. *Proceedings of the National Academy of Sciences*. 2002;99(11):7461-7466. doi:10.1073/pnas.102596199
45. Hogan PG, Rao A. Store-operated calcium entry: Mechanisms and modulation. *Biochem Biophys Res Commun*. 2015;460(1):40-49. doi:10.1016/j.bbrc.2015.02.110
46. Huang GN, Zeng W, Kim JY, et al. STIM1 carboxyl-terminus activates native SOC, Icrac and TRPC1 channels. *Nat Cell Biol*. 2006;8(9):1003-1010. doi:10.1038/ncb1454
47. Human Protein Atlas. TRPC3-Expression im Pankreas. Verfügbar unter: https://www.proteinatlas.org/ENSG00000138741-TRPC3/tissue/pancreas. Zugriffsdatum: 30. April 2025.
48. Human Protein Atlas. TRPC3-Expression in der Nebenschilddrüse. Verfügbar unter: https://www.proteinatlas.org/ENSG00000138741-TRPC3/tissue/parathyroid+gland. Zugriffsdatum: 30.April 2025.
49. Human Protein Atlas. TRPC3-Expression in der Schilddrüse. Verfügbar unter: https://www.proteinatlas.org/ENSG00000138741-TRPC3/tissue/thyroid+gland. Zugriffsdatum: 30.April 2025.
50. Human Protein Atlas. TRPC6-Expression im Pankreas. Verfügbar unter: https://www.proteinatlas.org/ENSG00000137672-TRPC6/tissue/pancreas. Zugriffsdatum: 30. April 2025.
51. Human Protein Atlas. TRPC6-Expression in der Nebenschilddrüse. Verfügbar unter: https://www.proteinatlas.org/ENSG00000137672-TRPC6/tissue/parathyroid+gland. Zugriffsdatum: 30. April 2025.
52. Human Protein Atlas. TRPC6-Expression in der Schilddrüse. Verfügbar unter: https://www.proteinatlas.org/ENSG00000137672-TRPC6/tissue/thyroid+gland. Zugriffsdatum: 30. April 2025.

53. Ibeh CL, Yiu AJ, Kanaras YL, et al. Evidence for a regulated Ca2+ entry in proximal tubular cells and its implication in calcium stone formation. *J Cell Sci*. 2019;132(9). doi:10.1242/jcs.225268
54. Jardin I, Gómez LJ, Salido GM, Rosado JA. Dynamic interaction of hTRPC6 with the Orai1–STIM1 complex or hTRPC3 mediates its role in capacitative or non-capacitative Ca2+ entry pathways. *Biochemical Journal*. 2009;420(2):267-277. doi:10.1042/BJ20082179
55. Kaneko Y, Szallasi A. Transient receptor potential (<scp>TRP</scp>) channels: a clinical perspective. *Br J Pharmacol*. 2014;171(10):2474-2507. doi:10.1111/bph.12414
56. Kashio M, Tominaga M. TRP channels in thermosensation. *Curr Opin Neurobiol*. 2022;75:102591. doi:10.1016/j.conb.2022.102591
57. Khan M, Jose A, Sharma S. *Physiology, Parathyroid Hormone*.; 2025.
58. Kim JY, Zeng W, Kiselyov K, et al. Homer 1 Mediates Store- and Inositol 1,4,5-Trisphosphate Receptor-dependent Translocation and Retrieval of TRPC3 to the Plasma Membrane. *Journal of Biological Chemistry*. 2006;281(43):32540-32549. doi:10.1074/jbc.M602496200
59. Kim MS, Hong JH, Li Q, et al. Deletion of TRPC3 in Mice Reduces Store-Operated Ca2+ Influx and the Severity of Acute Pancreatitis. *Gastroenterology*. 2009;137(4):1509-1517. doi:10.1053/j.gastro.2009.07.042
60. Kirstein E, Diebolt CM, Wagner M, et al. Distribution of TRPC1, TRPC3, and TRPC6 in the human thyroid. *Pathol Res Pract*. 2025;266:155796. doi:10.1016/j.prp.2024.155796
61. Kirstein E, Schaudien D, Wagner M, et al. TRPC3 Is Downregulated in Primary Hyperparathyroidism. *Int J Mol Sci*. 2024;25(8):4392. doi:10.3390/ijms25084392
62. Leung PS. Overview of the Pancreas. In: ; 2010:3-12. doi:10.1007/978-90-481-9060-7_1
63. Loevner LA. Imaging of the thyroid gland. *Seminars in Ultrasound, CT and MRI*. 1996;17(6):539-562. doi:10.1016/S0887-2171(96)90003-7
64. Lu M, Bränström R, Berglund E, et al. Expression and association of TRPC subtypes with Orai1 and STIM1 in human parathyroid. *J Mol Endocrinol*. 2010;44(5):285-294. doi:10.1677/JME-09-0138
65. Lu M, Fang X xia, Shi D dan, et al. A Selective TRPC3 Inhibitor Pyr3 Attenuates Myocardial Ischemia/Reperfusion Injury in Mice. *Curr Med Sci*. 2020;40(6):1107-1113. doi:10.1007/s11596-020-2293-y

66. Maier T, Follmann M, Hessler G, et al. Discovery and pharmacological characterization of a novel potent inhibitor of diacylglycerol-sensitive <scp>TRPC</scp> cation channels. *Br J Pharmacol*. 2015;172(14):3650-3660. doi:10.1111/bph.13151
67. Marabita F, Islam MdS. Expression of Transient Receptor Potential Channels in the Purified Human Pancreatic β-Cells. *Pancreas*. 2017;46(1):97-101. doi:10.1097/MPA.0000000000000685
68. Masson B, Saint-Martin Willer A, Dutheil M, et al. Contribution of transient receptor potential canonical channels in human and experimental pulmonary arterial hypertension. *American Journal of Physiology-Lung Cellular and Molecular Physiology*. 2023;325(2):L246-L261. doi:10.1152/ajplung.00011.2023
69. Miller BA. TRPC2. In: ; 2014:53-65. doi:10.1007/978-3-642-54215-2_3
70. Minisola S, Arnold A, Belaya Z, et al. Epidemiology, Pathophysiology, and Genetics of Primary Hyperparathyroidism. *Journal of Bone and Mineral Research*. 2022;37(11):2315-2329. doi:10.1002/jbmr.4665
71. Mohebati A, Shaha AR. Anatomy of thyroid and parathyroid glands and neurovascular relations. *Clinical Anatomy*. 2012;25(1):19-31. doi:10.1002/ca.21220
72. Mukerji N, Damodaran T V., Winn MP. TRPC6 and FSGS: The latest TRP channelopathy. *Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease*. 2007;1772(8):859-868. doi:10.1016/j.bbadis.2007.03.005
73. Müller M, Walkling J, Seemann N, Rustenbeck I. The Dynamics of Calcium Signaling in Beta Cells—A Discussion on the Comparison of Experimental and Modelling Data. *Int J Mol Sci*. 2023;24(4):3206. doi:10.3390/ijms24043206
74. Nilius B, Owsianik G. The transient receptor potential family of ion channels. *Genome Biol*. 2011;12(3):218. doi:10.1186/gb-2011-12-3-218
75. Numaga-Tomita T, Nishida M. TRPC Channels in Cardiac Plasticity. *Cells*. 2020;9(2):454. doi:10.3390/cells9020454
76. Ong HL, de Souza LB, Cheng KT, Ambudkar IS. Physiological Functions and Regulation of TRPC Channels. In: ; 2014:1005-1034. doi:10.1007/978-3-319-05161-1_12
77. Onohara N, Nishida M, Inoue R, et al. TRPC3 and TRPC6 are essential for angiotensin II-induced cardiac hypertrophy. *EMBO J*. 2006;25(22):5305-5316. doi:10.1038/sj.emboj.7601417
78. Onopiuk M, Eby B, Nesin V, et al. Control of PTH secretion by the TRPC1 ion channel. *JCI Insight*. 2020;5(8). doi:10.1172/jci.insight.132496
79. Patergnani S, Danese A, Bouhamida E, et al. Various Aspects of Calcium Signaling in the Regulation of Apoptosis, Autophagy, Cell Proliferation, and Cancer. *Int J Mol Sci*. 2020;21(21):8323. doi:10.3390/ijms21218323

80. Pedersen SF, Owsianik G, Nilius B. TRP channels: An overview. *Cell Calcium*. 2005;38(3-4):233-252. doi:10.1016/j.ceca.2005.06.028
81. Pirahanchi Y, Toro F, Jialal I. *Physiology, Thyroid Stimulating Hormone*.; 2023.
82. Putney JW. Pharmacology of Store-operated Calcium Channels. *Mol Interv*. 2010;10(4):209-218. doi:10.1124/mi.10.4.4
83. Rached G, Saliba Y, Maddah D, et al. TRPC3 Regulates Islet Beta-Cell Insulin Secretion. *Advanced Science*. 2023;10(6). doi:10.1002/advs.202204846
84. Ramsey IS, Delling M, Clapham DE. AN INTRODUCTION TO TRP CHANNELS. *Annu Rev Physiol*. 2006;68(1):619-647. doi:10.1146/annurev.physiol.68.040204.100431
85. Riccio A, Medhurst AD, Mattei C, et al. mRNA distribution analysis of human TRPC family in CNS and peripheral tissues. *Molecular Brain Research*. 2002;109(1-2):95-104. doi:10.1016/S0169-328X(02)00527-2
86. Rorsman P, Ashcroft FM. Pancreatic β-Cell Electrical Activity and Insulin Secretion: Of Mice and Men. *Physiol Rev*. 2018;98(1):117-214. doi:10.1152/physrev.00008.2017
87. Rossier MF. T-Type Calcium Channel: A Privileged Gate for Calcium Entry and Control of Adrenal Steroidogenesis. *Front Endocrinol (Lausanne)*. 2016;7. doi:10.3389/fendo.2016.00043
88. Seo K, Rainer PP, Shalkey Hahn V, et al. Combined TRPC3 and TRPC6 blockade by selective small-molecule or genetic deletion inhibits pathological cardiac hypertrophy. *Proceedings of the National Academy of Sciences*. 2014;111(4):1551-1556. doi:10.1073/pnas.1308963111
89. Shahid Z, Singh G. *Physiology, Islets of Langerhans*.; 2025.
90. Srinivasan A, Wong FK, Karponis D. Calcitonin: A useful old friend. *J Musculoskelet Neuronal Interact*. 2020;20(4):600-609.
91. Staruschenko A, Ma R, Palygin O, Dryer SE. Ion channels and channelopathies in glomeruli. *Physiol Rev*. 2023;103(1):787-854. doi:10.1152/physrev.00013.2022
92. STOJILKOVIC SS. Pituitary cell type-specific electrical activity, calcium signaling and secretion. *Biol Res*. 2006;39(3). doi:10.4067/S0716-97602006000300004
93. Strowski MZ, Blake AD. Function and expression of somatostatin receptors of the endocrine pancreas. *Mol Cell Endocrinol*. 2008;286(1-2):169-179. doi:10.1016/j.mce.2008.02.007
94. Sukumaran P, Löf C, Pulli I, Kemppainen K, Viitanen T, Törnquist K. Significance of the transient receptor potential canonical 2 (TRPC2) channel in the regulation of rat thyroid FRTL-5 cell proliferation, migration, adhesion and invasion. *Mol Cell Endocrinol*. 2013;374(1-2):10-21. doi:10.1016/j.mce.2013.03.026

95. Tang Q, Guo W, Zheng L, et al. Structure of the receptor-activated human TRPC6 and TRPC3 ion channels. *Cell Res*. 2018;28(7):746-755. doi:10.1038/s41422-018-0038-2
96. Thompson JL, Shuttleworth TJ. Exploring the unique features of the ARC channel, a Store-independent Orai channel. *Channels*. 2013;7(5):364-373. doi:10.4161/chan.26156
97. Umlauf F, Diebolt CM, Englisch CN, Flockerzi F, Tschernig T. Distribution of TRPC5 in the human lung: A study in body donors. *Exp Ther Med*. 2024;28(3):363. doi:10.3892/etm.2024.12652
98. Unger RH, Cherrington AD. Glucagonocentric restructuring of diabetes: a pathophysiologic and therapeutic makeover. *Journal of Clinical Investigation*. 2012;122(1):4-12. doi:10.1172/JCI60016
99. Vazquez G, Wedel BJ, Aziz O, Trebak M, Putney JW. The mammalian TRPC cation channels. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research*. 2004;1742(1-3):21-36. doi:10.1016/j.bbamcr.2004.08.015
100. Venkatachalam K, Montell C. TRP Channels. *Annu Rev Biochem*. 2007;76(1):387-417. doi:10.1146/annurev.biochem.75.103004.142819
101. Wang H, Cheng X, Tian J, et al. TRPC channels: Structure, function, regulation and recent advances in small molecular probes. *Pharmacol Ther*. 2020;209:107497. doi:10.1016/j.pharmthera.2020.107497
102. Watanabe H, Murakami M, Ohba T, Takahashi Y, Ito H. TRP channel and cardiovascular disease. *Pharmacol Ther*. 2008;118(3):337-351. doi:10.1016/j.pharmthera.2008.03.008
103. Whitcomb DC, Lowe ME. Human Pancreatic Digestive Enzymes. *Dig Dis Sci*. 2007;52(1):1-17. doi:10.1007/s10620-006-9589-z
104. Yamada H, Yoshida M, Ito K, et al. Potentiation of Glucose-stimulated Insulin Secretion by the GPR40–PLC–TRPC Pathway in Pancreatic β-Cells. *Sci Rep*. 2016;6(1):25912. doi:10.1038/srep25912
105. Yamamura H, Suzuki Y, Yamamura A. Pathophysiological roles of TRPC6 channels in pulmonary arterial hypertension. *Folia Pharmacologica Japonica*. 2020;155(4):230-235. doi:10.1254/fpj20001
106. Zernov N, Popugaeva E. Role of Neuronal TRPC6 Channels in Synapse Development, Memory Formation and Animal Behavior. *Int J Mol Sci*. 2023;24(20):15415. doi:10.3390/ijms242015415

6. Publikationsverzeichnis

Publikationen im Zusammenhang mit der Dissertation

1. Kirstein E, Schaudien D, Wagner M, Diebolt CM, Bozzato A, Tschernig T, Englisch CN. TRPC3 Is Downregulated in Primary Hyperparathyroidism. Int J Mol Sci. 2024 Apr 16;25(8):4392. doi: 10.3390/ijms25084392. PMID: 38673977; PMCID: PMC11049814.

2. Kirstein E, Diebolt CM, Wagner M, Bozzato A, Federspiel JM, Schaudien D, Tschernig T, Englisch CN. Distribution of TRPC1, TRPC3, and TRPC6 in the human thyroid. Pathol Res Pract. 2024 Dec 24;266:155796. doi: 10.1016/j.prp.2024.155796. Epub ahead of print. PMID: 39740284.

3. Englisch CN, Kirstein E, Diebolt CM, Wagner M, Tschernig T. Distribution of TRPC3 and TRPC6 in the human exocrine and endocrine pancreas. Pathol Res Pract. 2024 Aug;260:155403. doi: 10.1016/j.prp.2024.155403. Epub 2024 Jun 10. PMID: 38870712.

Weitere Publikationen

1. Englisch CN, Diebolt CM, Schaudien D, Klamminger GG, Kirstein E, Junker K, Wagner M, Tschernig T. Proximal Tubular TRPC3 Immunostaining Is Reduced in Human Nephrocalcinosis. Lab Invest. 2024 Sep;104(9):102109. doi: 10.1016/j.labinv.2024.102109. Epub 2024 Aug 1. PMID: 39094476.

2. Englisch CN, Diebolt CM, Kirstein E, Wahl V, Wartenberg P, Schaudien D, Beckmann A, Laschke MW, Krasteva-Christ G, Gudermann T, Chubanov V, Boehm U, Tschernig T. TRPM6 in murine kidneys-of targets and antibodies. Naunyn Schmiedebergs Arch Pharmacol. 2025 Mar 1. doi: 10.1007/s00210-025-03951-0. Epub ahead of print. PMID: 40025338.

7. Abbildungsverzeichnis

Sämtliche Abbildungen in dieser Dissertation wurden mit der Plattform **BioRender.com** erstellt. Die verwendeten Elemente und Vorlagen unterliegen den Nutzungsbedingungen von BioRender und wurden im Rahmen einer gültigen Publication License verwendet. Zugriff am [23.05.2025], Agreement Number: AG-CV28AVYBQV (Abbildung 1), AG-MT28AVYDWD (Abbildung 2), AG-PD28AVYJ1R (Abbildung 3), AG-UY28AVXZBC (Abbildung 4), AG-ZI28AVYN82 (Abbildung 5), AG-VO28AVYKTE (Abbildung 6).

8. Danksagung

Mein besonderer Dank gilt meinem Doktorvater Prof. Dr. med. Thomas Tschernig, der mich mit großer Expertise, wertvollen Anregungen und konstruktivem Feedback auf diesem wissenschaftlichen Weg begleitet hat. Seine Unterstützung und sein Vertrauen haben maßgeblich zu dieser Arbeit beigetragen.

Ebenso danke ich meinem Betreuer und guten Freund Colya Englisch, der mich nicht nur fachlich mit Rat und Tat unterstützt hat, sondern auch durch unzählige Diskussionen, gemeinsames Tüfteln und seine Freundschaft eine große Stütze war.

Ein ebenso herzliches Dankeschön geht an Coline Diebolt, die mir vor allem im Labor stets mit Rat, Tat und guter Laune zur Seite stand. Ihre Unterstützung war für den praktischen Teil dieser Arbeit unersetzlich – und ihre Freundschaft hat diesen Weg umso bereichernder gemacht.

Dem Team der Prosektur des Anatomischen Institutes der Universität des Saarlandes danke ich für die enge Zusammenarbeit, die wertvolle Unterstützung und das stets offene Ohr. Mein Dank gilt insbesondere Irina Scheck und Katja Schäfer, die mit ihrer Expertise und Hilfsbereitschaft eine entscheidende Rolle gespielt haben.

Für die zur Verfügung gestellten Gewebeproben und den wertvollen Input danke ich Prof. Dr. med. Mathias Wagner aus dem Pathologischen Institut der Universität des Saarlandes, Prof. Dr. med. Alessandro Bozzato aus der Klinik für Hals-Nasen-Ohrenheilkunde am Universitätsklinikum des Saarlandes, sowie Dr. Dirk Schaudien aus dem Fraunhofer Institut für Toxikologie und Experimentelle Medizin in Hannover. Ein besonderer Dank gebührt Dr. Dirk Schaudien für seine fachliche Unterstützung, sowie die Ressourcen des Instituts, die maßgeblich zur statistischen Analyse sowie zur Digitalisierung der Proben beigetragen haben.

Nicht zuletzt danke ich von Herzen Familie und Freunden, die mich über all die Jahre mit unermüdlichem Rückhalt, Geduld und Motivation begleitet haben. Ihre Unterstützung – sei es durch aufmunternde Worte, geduldiges Zuhören oder einfach ihr Dasein – hat mich sehr bereichert!

Diese Arbeit wäre ohne Kooperationen vieler Köpfe, Hände und Herzen nicht möglich gewesen. Danke euch allen!

9. Lebenslauf

Aus datenschutzrechtlichen Gründen wird der Lebenslauf in der elektronischen Fassung der Dissertation nicht veröffentlicht.

Aus datenschutzrechtlichen Gründen wird der Lebenslauf in der elektronischen Fassung der Dissertation nicht veröffentlicht.

Aus datenschutzrechtlichen Gründen wird der Lebenslauf in der elektronischen Fassung der Dissertation nicht veröffentlicht.