Visualization of Kallikreins in Thyroid Epithelial and Carcinoma Cells

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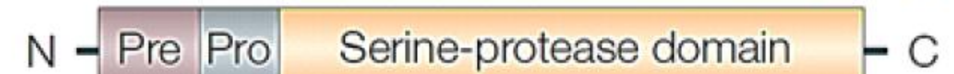


INTRODUCTION

Kallikrein expression in thyroid tissue (transcriptional level by ISH and RT-PCR)

KLK2 - KLK3/PSA - KLK4 - KLK5 - KLK7 - KLK11 - KLK12 - KLK13 - KLK14 - KLK15

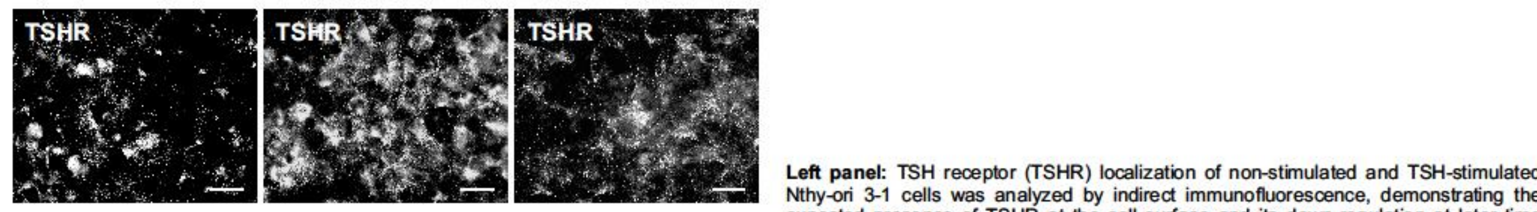
Kallikreins (KLKs) are a family of hormonally regulated proteinases comprising 15 related enzymes that are expressed in many tissues and cell types. The aim of this study is to gain insights into their localization in thyroid epithelial and carcinoma cells and trafficking upon thyroid stimulating hormone (TSH) treatment. Here, we focused on KLK2, KLK3/PSA (prostate-specific antigen), and KLK7, and studied their trafficking in non-stimulated and TSH-treated thyroid epithelial and carcinoma cells. In addition, synchronization studies were conducted to investigate *de novo* biosynthesis and transport pathways of KLKs during cell cycle progression.

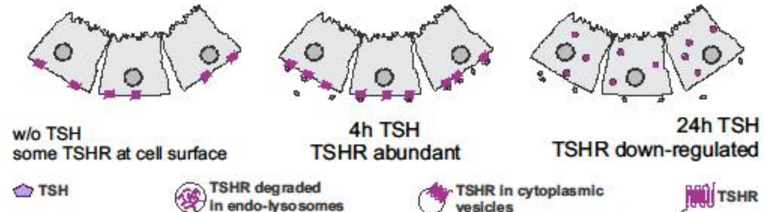
Molecular structure of tissue kallikreins (KLK)

N – Pre | Pro | Serine-protease domain – C

Schematic drawing depicting the molecular structure of KLKs which are synthesized as preproenzymes at the rough endoplasmic reticulum (rER), where the signal peptide (pre) targets for entry into the ER lumen while the N-terminal propeptide (pro) prevents premature proteolytic activity of the zymogen forms that are known to become activated upon secretion into the extracellular space.

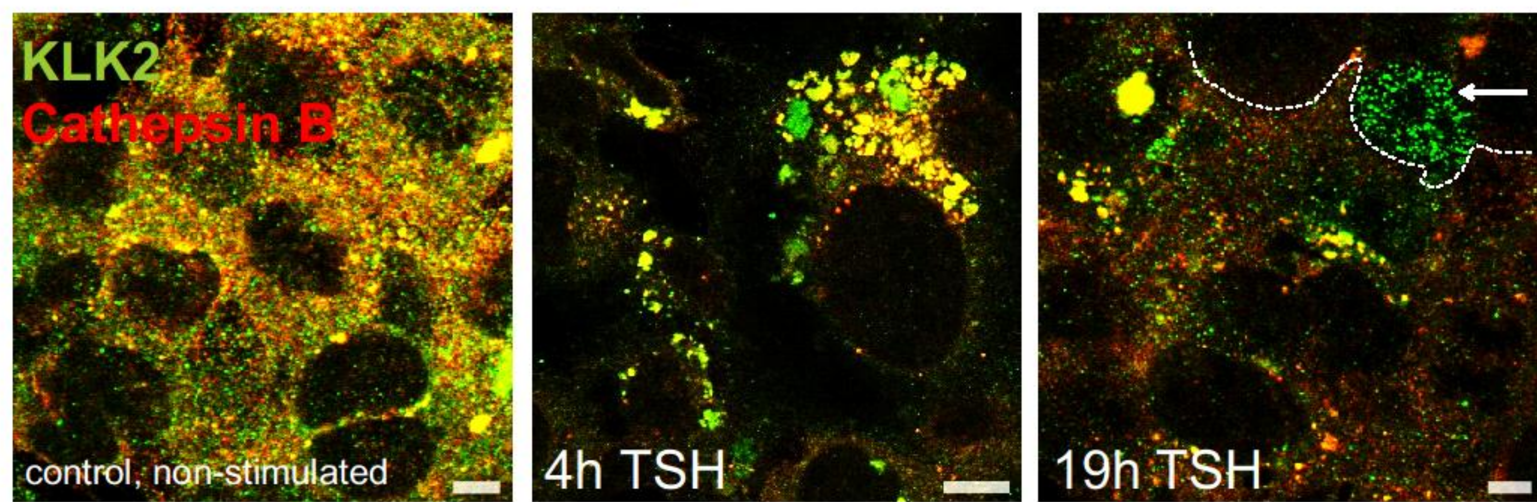
TSH stimulation of Nthy-ori 3-1 cells triggers secretion of kallikreins like KLK2 and KLK3/PSA



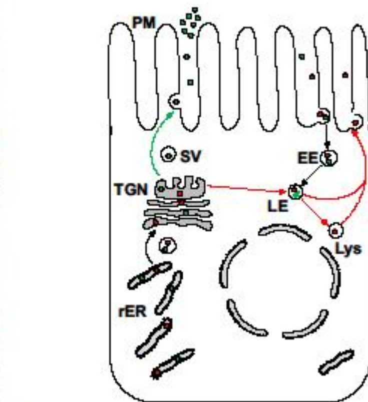


Left panel: TSH receptor (TSHR) localization of non-stimulated and TSH-stimulated Nthy-ori 3-1 cells was analyzed by indirect immunofluorescence, demonstrating the expected presence of TSHR at the cell surface and its down-regulation at later time intervals of stimulation.

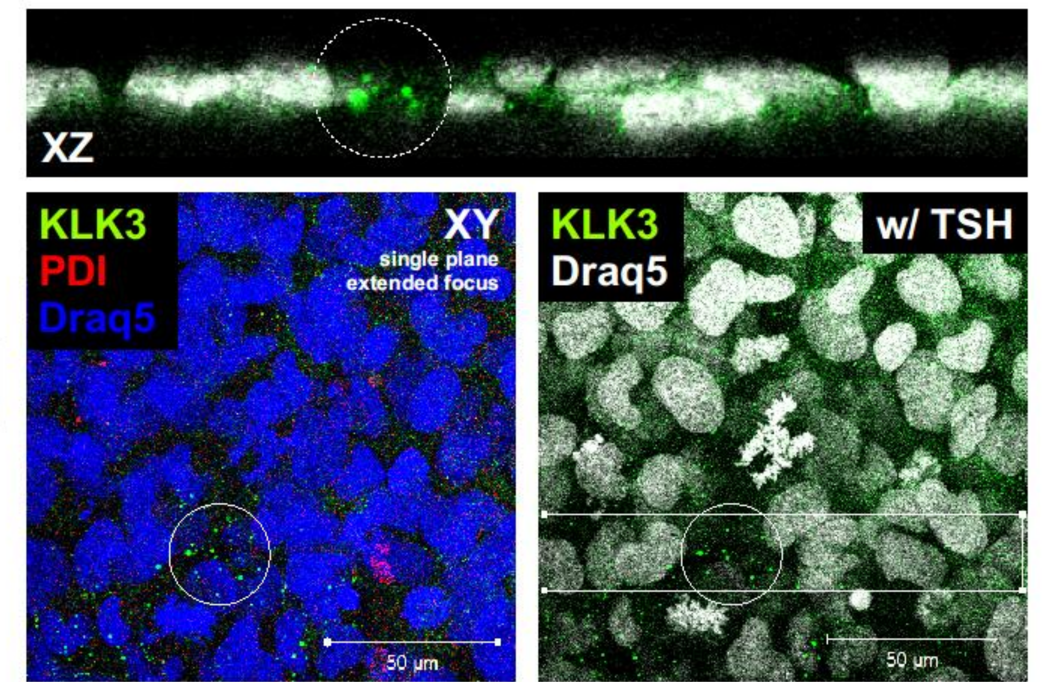
Bottom panel:
KLK2 (green) was present in reticular structures of non-stimulated Nthy-ori 3-1 cells, and partially co-localized with cysteine cathepsin B (yellow), an endo-lysosomal protease (red). Upon TSH stimulation, thyrocytes featured numerous endocytic vesicles in which KLK2 was co-localized with cathepsin B (4h TSH, yellow). KLK2 was also found to be secreted from the human thyroid epithelial cells and remained associated in the peri-cellular space (19h TSH, green).



Explanation for co-localization of KLKs and cysteine cathepsins in vesicles of the endocytic pathway



The schematic drawing illustrates how the transport pathways of kallikreins (green) differ and merge with cathepsin trafficking to endo-lysosomes (red).



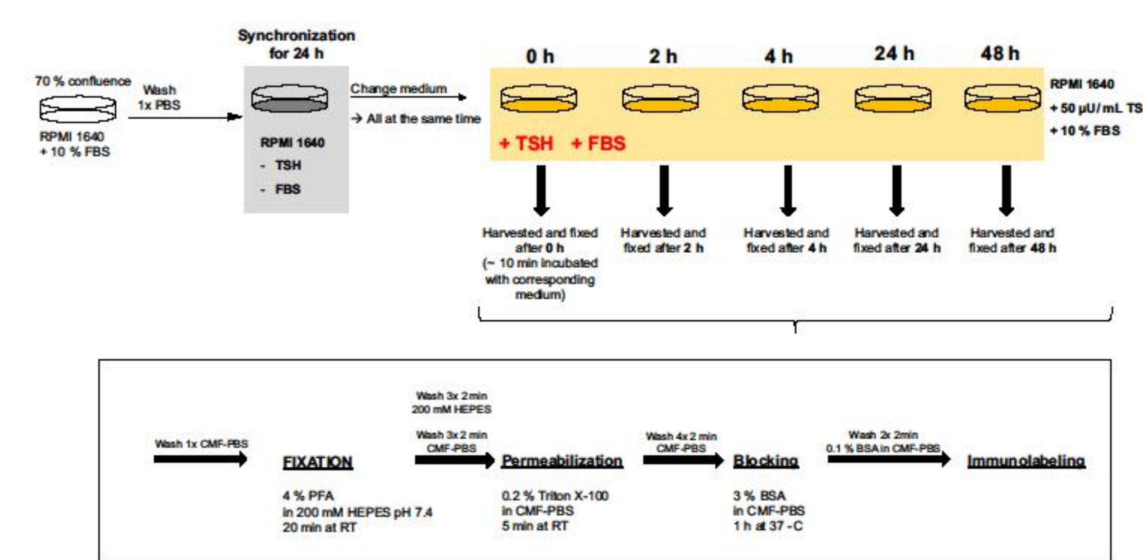
KLK3 is secreted from Nthy-ori 3-1 cells upon 4h TSH stimulation.

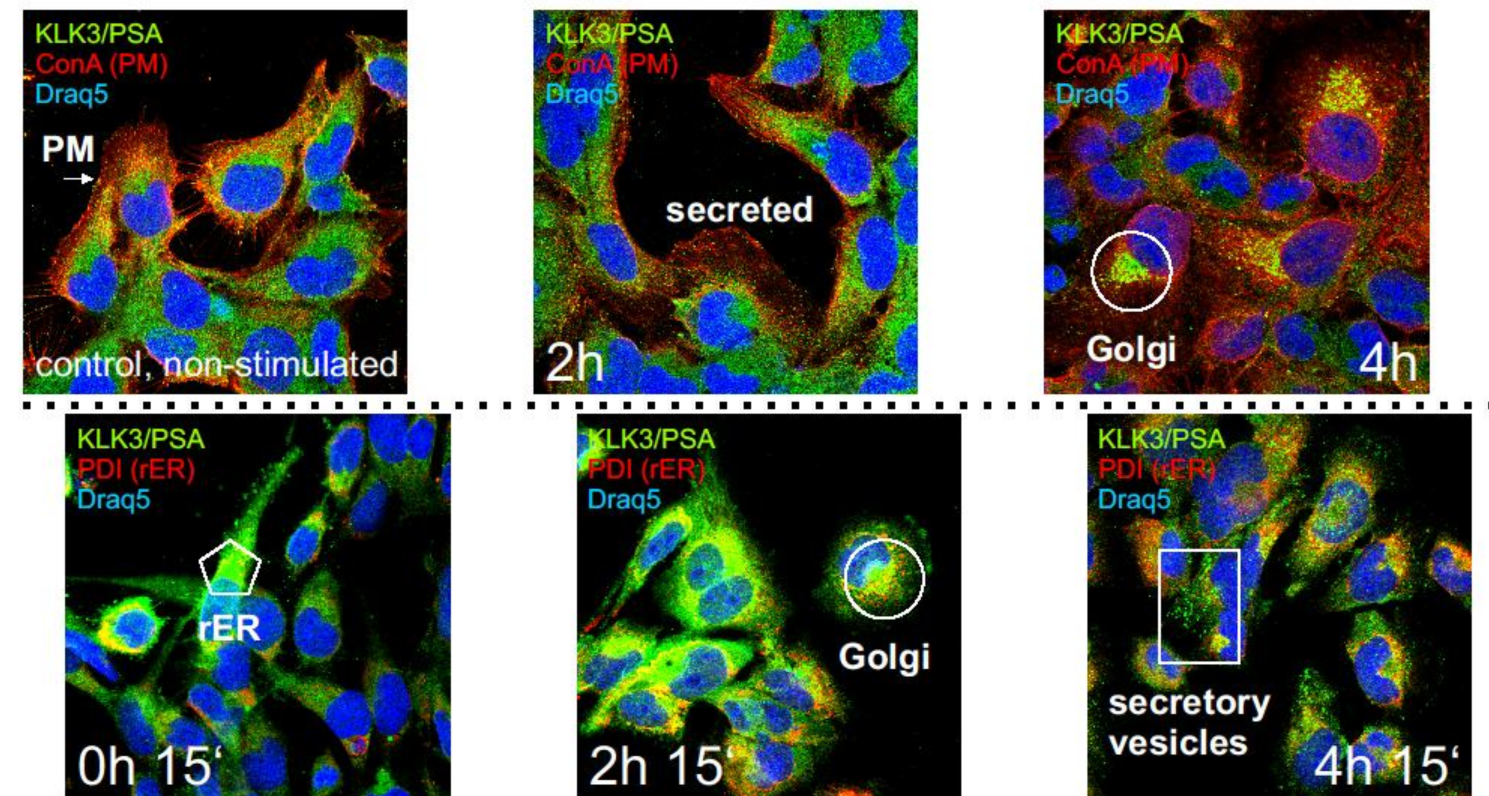
Confocal laser scanning micrographs of PFA-fixed and Triton X-100 permeabilized Nthy-ori 3-1 cells in confluent monolayers were immunolabeled with antibodies against KLK3/ PSA (green) and the ER-resident protein PDI (red). Immunostaining revealed that no co-localization of KLK3 and PDI was detectable 4h after TSH stimulation, indicating that KLK3/PSA followed the secretory pathway and was secreted from Nthy-ori 3-1 cells (circles).

Overlays of different channels and zero-projected images taken at different focal planes are depicted as indicated in the standard xy-presentation mode as an extended focus. The image on top is an extended focus of several sections with 25°-projection along xz, i.e. a tangential sectional view through the volume of the cells is displayed. Nuclei were counter-stained with Draq5 (blue and white signals); scale bars represent 50 µm.

Synchronization in G1-phase and subsequent TSH stimulation results in up-regulated *de novo* biosynthesis of KLK3

TSH-stimulation during release from cell cycle arrest in G1/G0-phase





KLK3 is co-localized with the ConA-positive glycocalyx components of Nthy-ori 3-1 cells in resting conditions, and its biosynthesis and transport along the secretory pathway is up-regulated upon TSH stimulation.

Nthy-ori 3-1 cells were incubated according to the scheme for synchronization in G1/G0-phase by serum-starvation, before the cultures were shifted out of the cell cycle arrest by further incubation in complete culture medium. TSH-stimulation was performed as indicated.

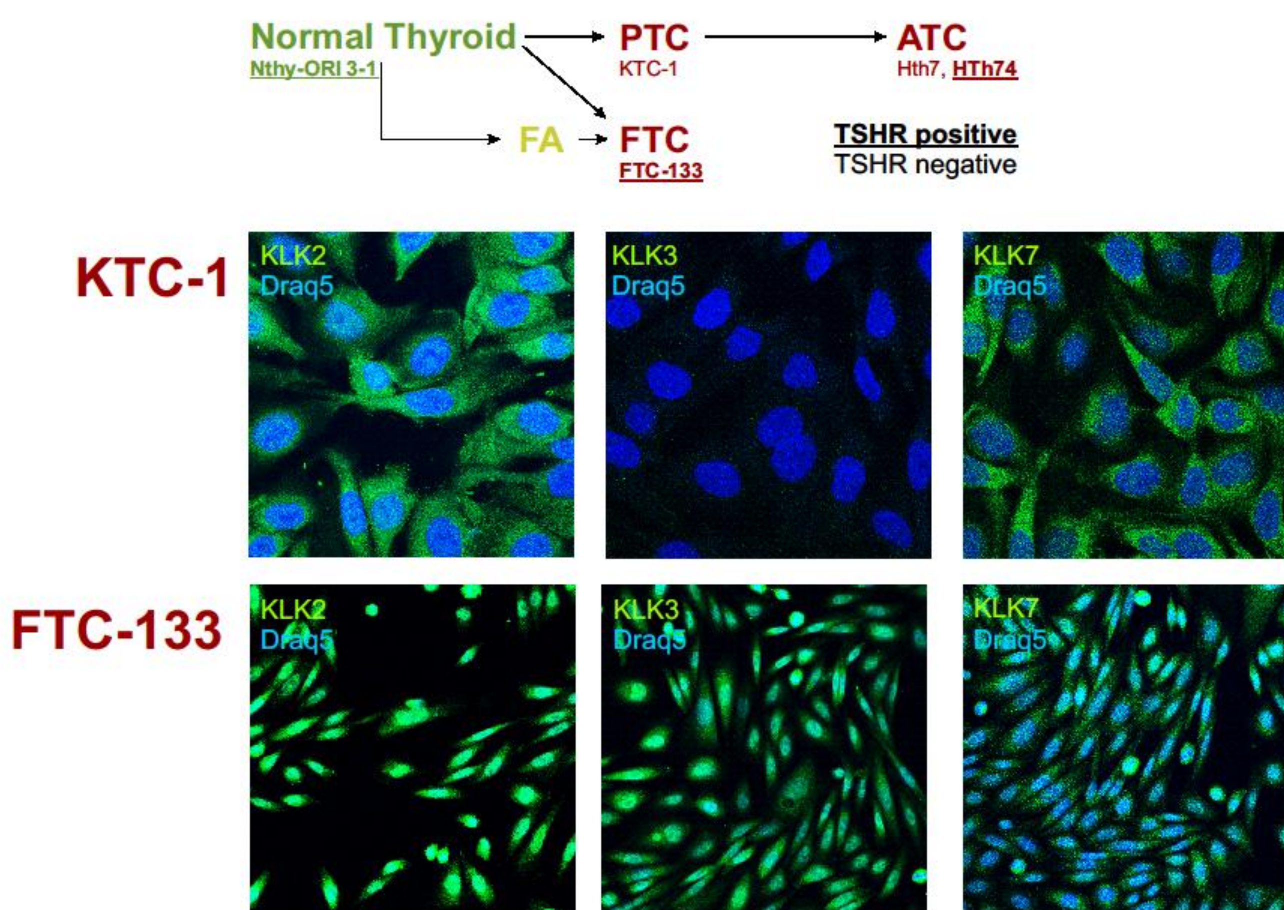
KLK3 is co-localized with the ConA-positive glycocalyx components of Nthy-ori 3-1 cells in resting conditions, and its biosynthesis and transport along the secretory pathway is up-regulated upon TSH stimulation.

Confocal laser scanning micrographs of Nthy-ori 3-1 cells immunolabeled with antibodies against KLK3/PSA (green), lectin staining with Concanavalin A (ConA, red), or immunostaining of the ER-resident chaperone protein disulfide isomerase (PDI), and counter-staining of nuclear DNA with Draq5 (blue).
Immunostaining revealed KLK3 in co-localization with ConA-positive glycocalyx components at the cell surface (PM) while also depicting the secreted protease in the extracellular space (secreted) upon short-term TSH-stimulation. At 4h of TSH-stimuation, KLK3/PSA was detected in the Golgi apparatus (Golgi, top panel).
Co-localization studies with the ER-resident PDI highlighted the trafficking of KLK3/PSA from the rER to the Golgi apparatus from where the protease was sorted into secretory vesicles (bottom panel).

Thyroid cell lines to analyze cancer states *in vitro*

Normal Thyroid (Nthy-ORI 3-1) → PTC (KTC-1) → ATC (Hth7, HTh74)
→ FA → FTC (FTC-133)

TSHR positive
TSHR negative



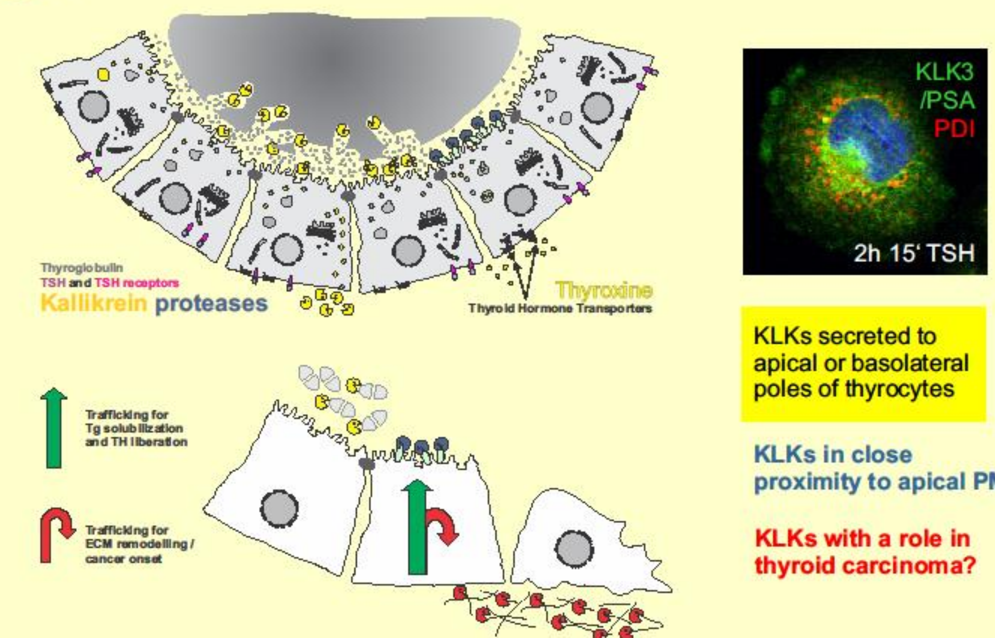
KLK2, KLK3/PSA, and KLK7 are differentially localized in papillary (PTC) and follicular thyroid carcinoma cells (FTC) with nuclear forms prevalent in particular in FTC.

Confocal laser scanning micrographs of KTC-1 and FTC-133 cells as indicated after immunolabeling with antibodies against KLK2, KLK3/PSA, or KLK7 (green), and counter-staining of nuclear DNA with Draq5 (blue).

Immunostaining demonstrated that KLK2 was detected in reticular and vesicular structures in the cytoplasm of KTC-1 and FTC-133 cells, while also being detectable in nuclei of the thyroid carcinoma cell lines. KLK3 was absent from KTC-1 cells while it was distributed as KLK2 in FTC-133 cells. In contrast, KLK7 was mainly localized to cytoplasmic compartments of the secretory pathway, and only occassionally detected in nuclei of the thyroid carcinoma cells.

The results indicated unexpected localization of KLKs in the FTC-133 and KTC-1 thyroid carcinoma cell lines, which requires further studies in order to decipher the molecular forms of KLKs reaching the nuclear compartment. We also aim at determining the proteolytic activity of nuclear forms of KLKs with specific activity based probes (ABPs).

CONCLUSION



Schematic representation of the observed localization of KLKs in thyroid epithelial anc carcinoma cells

In addition to their presence in compartments of the secretory and endocytic pathways, KLKs were localized to the extracellular space and in the peri-cellular region in close proximity to the apical plasma membrane of thyrocytes (yellow and blue symbols).

The presence of KLKs in extracellular compartments of thyroid tissue might indicate their secretion at the apical pole of the thyroid epithelium (green arrow) for subsequent proteolytic procession of thyroglobulin and thyroid hormone liberation from the precursor molecule, which was so far demonstrated for thyroid cysteine cathepsins [Friedrichs et al., 2003; Jordans et al., 2009; Dauth et al., 2011].

Basolateral secretion of KLKs (red arrow) proposes that they might also be involved in ECM degration thus contributing to cancer onset and progression. A novel function of KLKs is envisioned for specific forms detectable within the nuclei of thyroid carcinoma cells (pink symbols) [Brix et al., 2015].

References:

Friedrichs, B., C. Tepel, Th. Reinheckel, J. Deussing, K. von Figura, V. Herzog, Ch. Peters, P. Saftig, and K. Brix (2003). Thyroid functions of mouse cathepsins B, K, and L. J. Clin. Invest. 111, 1733-1745.
Jordans, S., S. Jenko-Kokalj, N.M. Kühl, S. Tedelind, W. Sendt, D. Brömme, D. Turk, and K. Brix (2009). Monitoring compartment-specific substrate cleavage by cathepsins B, K, L, and S at physiological pH and redox conditions. BMC Biochemistry 10, 23.
Dauth, S., M. Arampatzidou, M. Rehders, D.M.T. Yu, D. Führer, and K. Brix (2011). Thyroid cathepsin K – roles in physiology and thyroid disease. Clin. Rev. Bone Miner. Metab. 9, 94-106.
Brix, K., J. McInnes, A. Al-Hashimi, M. Rehders, T. Tamhane, M.H. Haugen (2015). Proteolysis mediated by cysteine cathepsins and legumain - recent advances and cell biological challenges. Protoplasma 252, 755-774

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