Nuclear Cysteine Cathepsins in Thyroid Carcinoma

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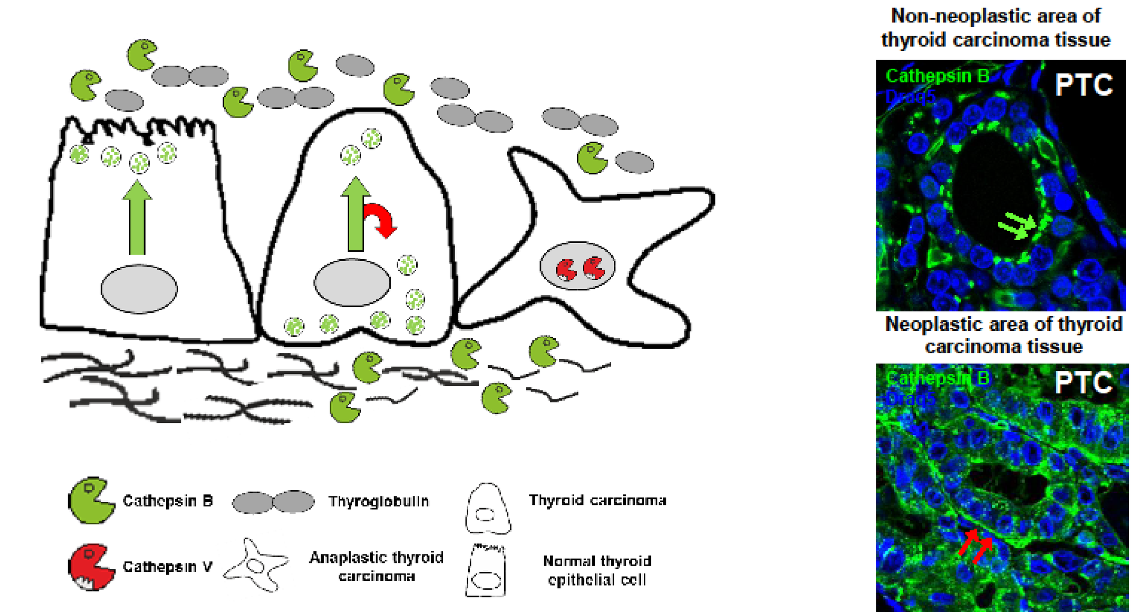
Introduction

Cysteine cathepsins play crucial roles in thyroid physiology through thyroglobulin processing for liberation of thyroid hormones by extra- and intracellular means. However, in thyroid cancer, cathepsin B is over-expressed and secreted into the extracellular space, thus promoting migratory phenotypes of thyroid carcinoma cells through excessive extracellular matrix degradation [1]. In addition, we have shown that N-terminally truncated forms of cathepsin V, which lack the signal peptide and parts of the pro-peptide, are localized to the nuclei of anaplastic thyroid carcinoma cells, while cathepsin L is present within endo-lysosomes as expected [1].

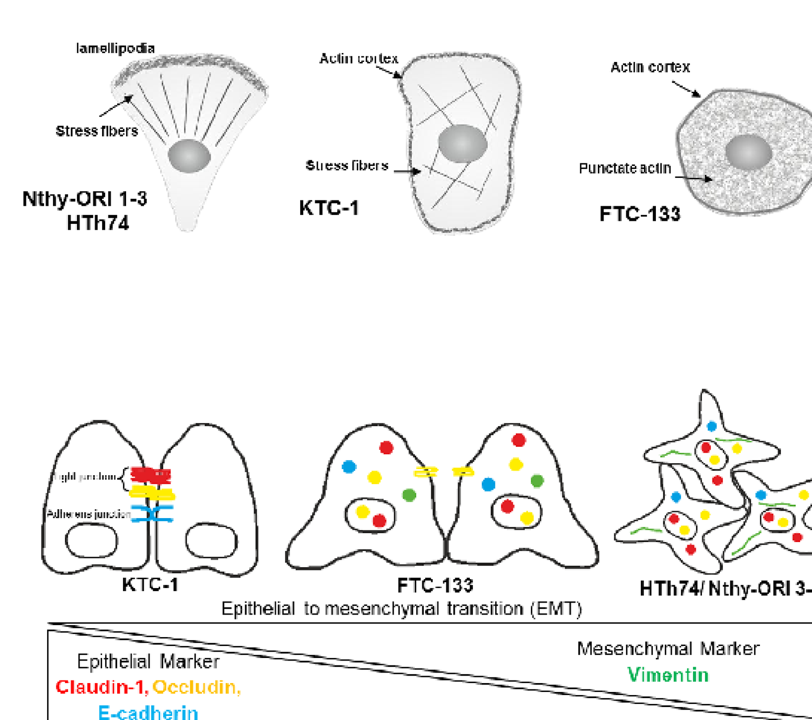
Here, localization patterns of cathepsins B and V were examined in a variety of human thyroid carcinoma cell lines in comparison to normal thyroid epithelial cells by immunofluorescence studies.

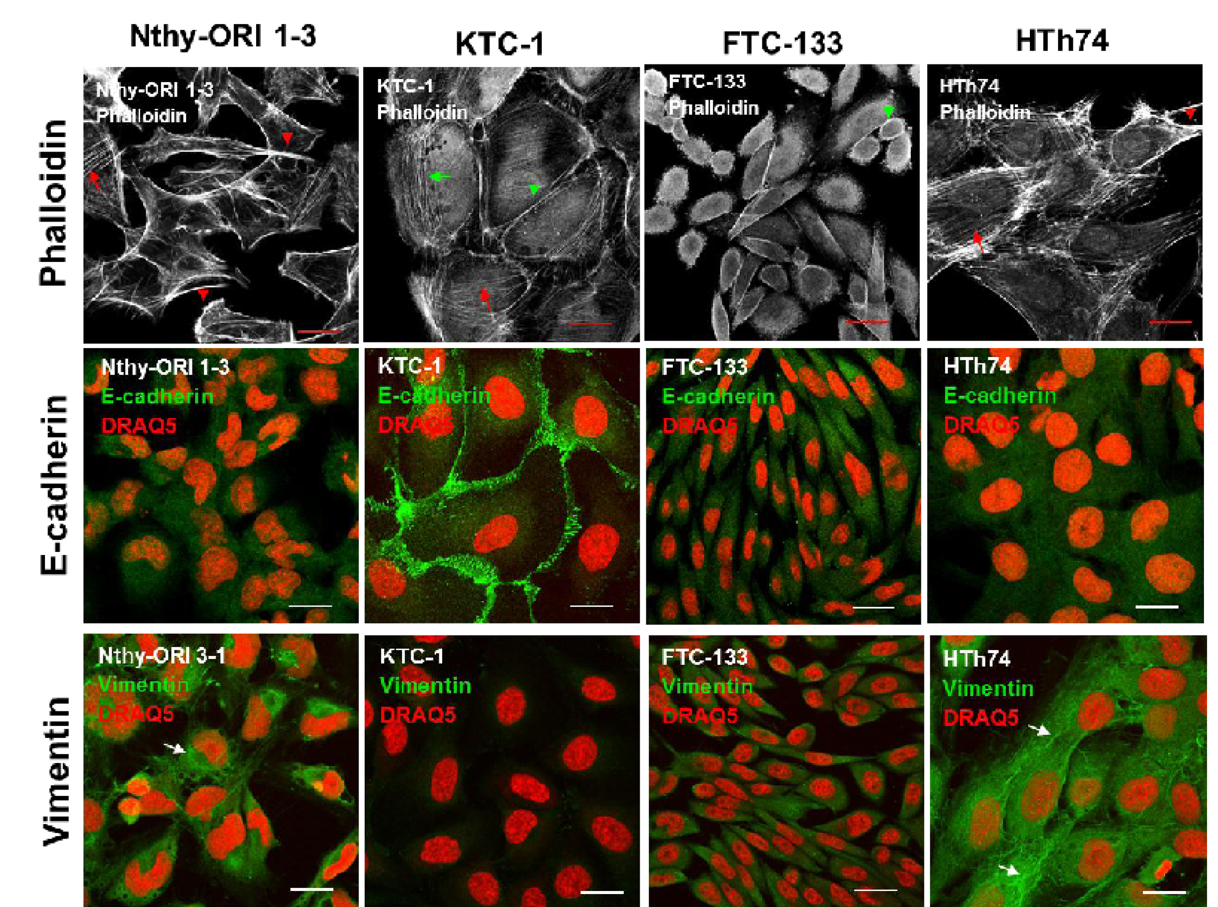


Characterization of Normal Thyroid Epithelial and Carcinoma Cell lines

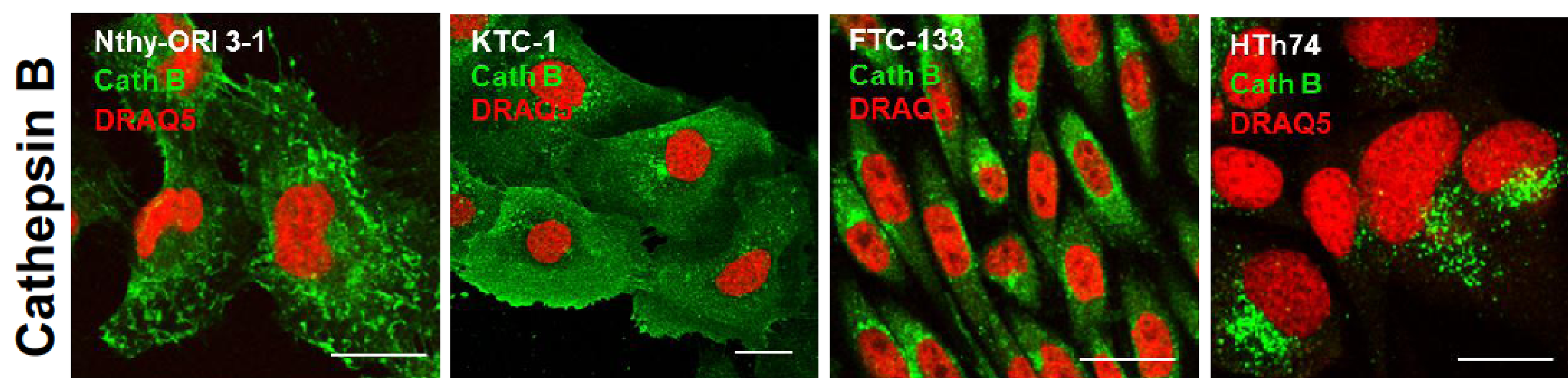
Nthy-ORI 3-1 and HTh74 cells labelled with FITC-phalloidin revealed stress fibers and lamellipodia, suggesting the ability of these cells to adhere to the substratum and to migrate, respectively. KTC-1 cells exhibited cortical F-actin, actin fibers, and a fine actin network. FTC-133 cells showed F-actin structures in the cytoplasm and prominent cortical F-actin, indicating that these cells lost their ability to tightly adhere to the extracellular matrix.

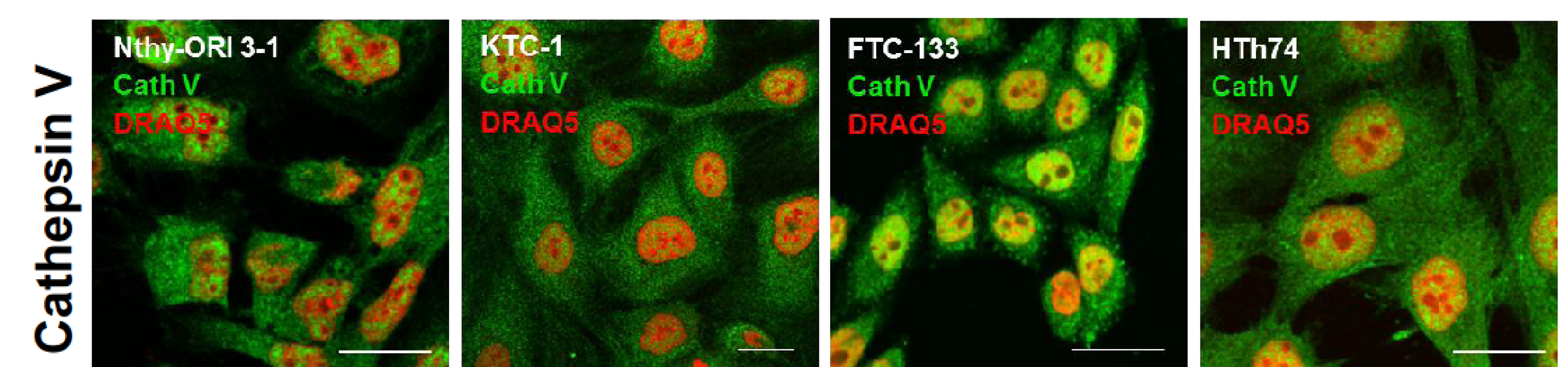
Nthy-ORI-3-1, KTC-1, FTC-133 and HTh74 cell lines represent different stages during epithelial-to-mesenchymal transition. KTC-1 cells displayed almost typical epithelial sheets composed of polarized cells. FTC-133 cells lacked cell-cell contacts and featured high levels of the mesenchymal marker vimentin. Mesenchymal characteristics including loss of intercellular adhesion and expression of vimentin was also observed in Nthy-ORI-3-1 and HTh74 cells. Nuclear-DNA was counter-stained with Draq5. Scale bars represent 20 µm.





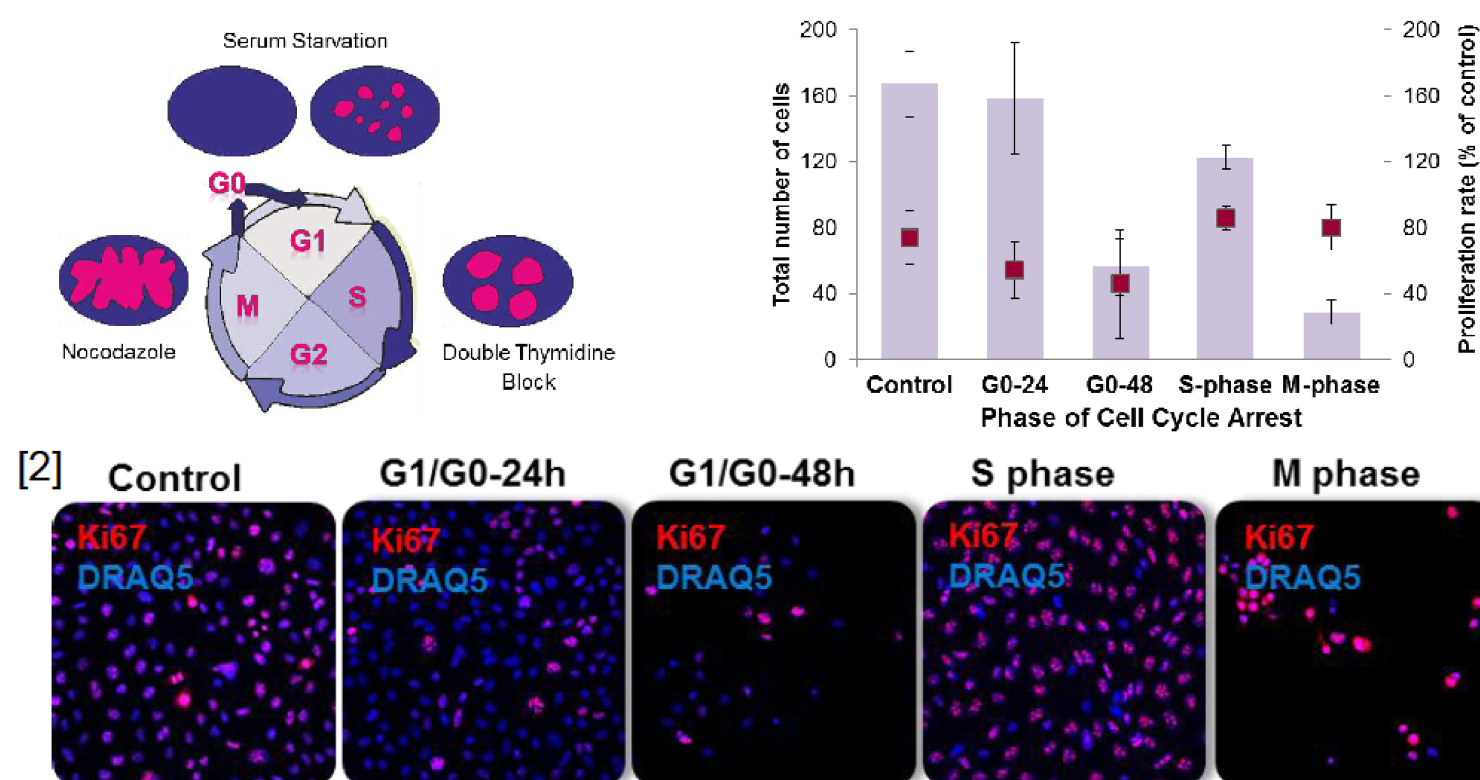
Localization of Cathepsins B and V



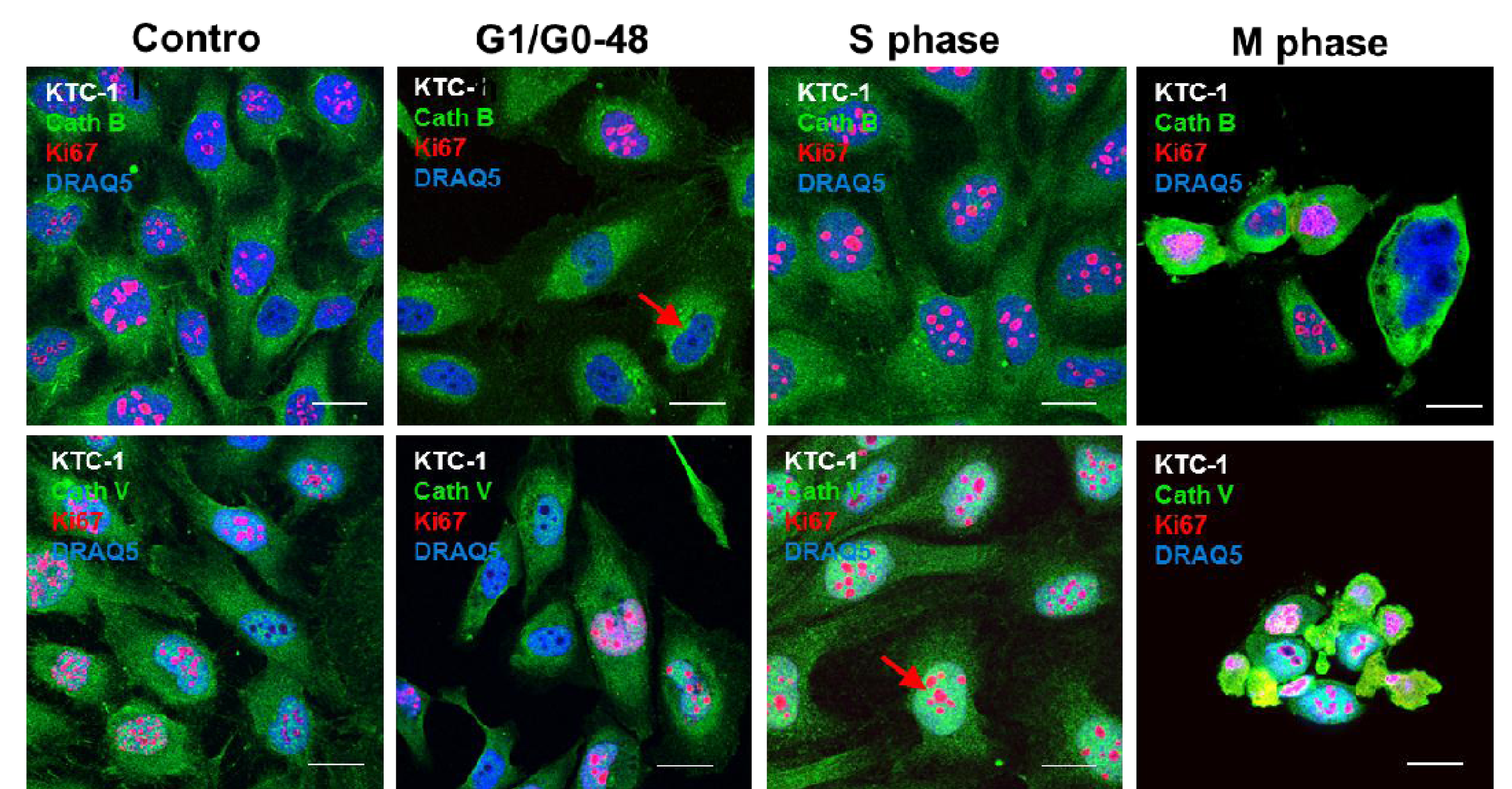


Human thyroid follicular epithelial cells (Nthy-ORI 3-1), human papillary (KTC-1), follicular (FTC-133) and, anaplastic (HTh74) thyroid carcinoma cell lines were stained with cathepsin B and V specific antibodies. Cathepsin B was found in reticular and vesicular structures distributed throughout the cytoplasm in Nthy-ORI 3-1, FTC-133, and KTC-1 cells, while vesicles containing cathepsin B were mainly accumulating in the peri-nuclear region of HTh74 cells. Immunostaining revealed that cathepsin V was prominently present in the nuclei of all four cell lines as well as in vesicles distributed throughout the cytoplasm. Nuclear-DNA was counter-stained with Draq5. Scale bars represent 20 µm.

Cathepsins B and V Localization During Cell Cycle of KTC-1 Cells



Arrest of KTC-1 cells in G1/G0 phase was by incubation in serum-free medium for 24 and 48 h. For synchronization in early S phase, cells were incubated in thymidine for 18 and 17 h interrupted by a 9-h-incubation in complete medium. For G2/M arrest, cells were treated with thymidine for 18 h, and released for 3 h by incubation in complete culture medium, before they were incubated with nocodazole for another 12 h. Cells were immunolabeled with Ki67 as proliferation marker, cathepsin B and V specific antibodies, and Draq5 counter-staining nuclear DNA.



Immunolabeling of synchronized KTC-1 cells revealed cathepsin B staining in ER-like reticular structures as well as in the Golgi apparatus and in endo-lysosomal vesicles, but no prominent nuclear staining was observed. In contrast, cathepsin V was also present in the nuclei throughout the cell cycle. In particular, proliferative cells featured nuclear cathepsin V signals. The strongest nuclear staining of cathepsin V was detected in the nuclei of KTC-1 cells in S phase.

Conclusions

- Human thyroid epithelial cells *in vitro* (Nthy-ORI 3-1) are less polarized than *in situ*.
- Cathepsin V rather than cathepsin B might serve important functions within the nuclei of thyroid carcinoma cells because it was more abundant in the nuclear compartment than cathepsin B.
- We hypothesize that cathepsin V is involved in de-regulation of cell cycle progression during thyroid tumorigenesis.

References

[1] Tedelind, S., et al., *Nuclear cysteine cathepsin variants in thyroid carcinoma cells.* Biol Chem, 2010. **391**(8): p. 923-35. [2] Tamhane, T., et al., *Nuclear cathepsin L activity is required for cell cycle progression of colorectal carcinoma cells.* Biochimie, 2016. **122**: p. 208-218.

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