Characterization of Novel Human Immortalized Thyroid Follicular Epithelial Cell Lines

Kristen Hopperstad[1], Theresa Truschel[2], Tom Wahlicht[2], Wendy Stewart[1], Andrew Eicher[1], Tobias May[2], Chad Deisenroth[1]

[1]Center for Computational Toxicology and Exposure, Office of Research and Development, U.S. Environmental Protection Agency, Research Triangle Park, NC 27709, United States
[2] InSCREENeX GmbH, Inhoffenstraße 7, 38124 Braunschweig, Germany

www.epa.gov

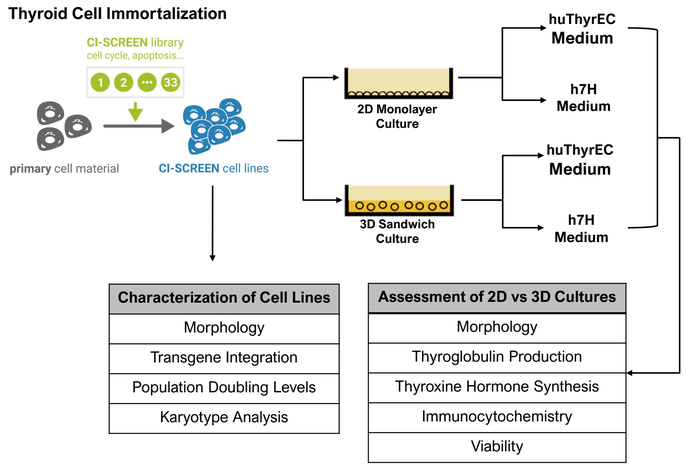
Kristen Hopperstad | hopperstad.kristen@epa.gov | 919-541-3743

Objective

Develop and characterize human thyroid cell lines capable of retaining key phenotypic features of the native thyroid gland.

- Investigation of normal human thyroid function using *in vitro* culture systems is dependent on cells that recapitulate physiology of differentiated thyrocytes.
- Primary thyrocytes retain features of the native organ but have limited lifespan in culture.
- Immortalized thyrocytes offer a sustainable and reproducible cellular resource if challenges maintaining phenotypic stability can be overcome to retain functional features of primary cells.

Study Design

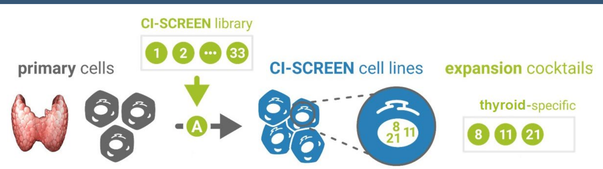


Characterization of Cell Lines
Morphology
Transgene Integration
Population Doubling Levels
Karyotype Analysis

Assessment of 2D vs 3D Cultures
Morphology
Thyroglobulin Production
Thyroxine Hormone Synthesis
Immunocytochemistry
Viability

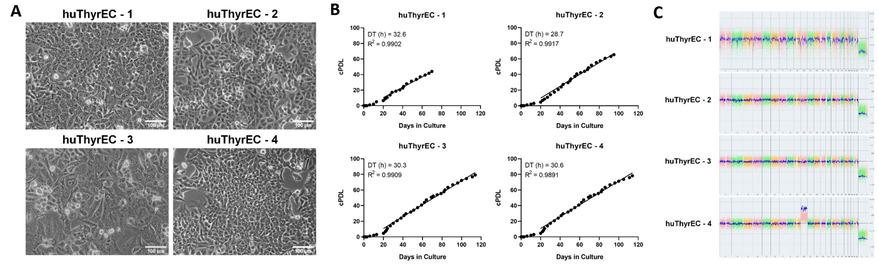
Overview of model characterization. Intact thyroid glands were derived from a single primary human donor. huThyrEc cell lines were generated by transduction of a lentiviral gene library composed of 33 different genes. Cell line expansion, maintenance, and passaging was performed in huThyrEC or h7H medium in 2D or 3D format for structural and functional analysis of key phenotypic features.

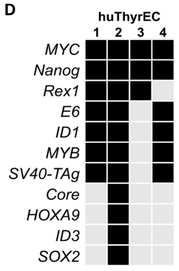
Human Thyrocyte Immortalization



The InSCREENeX CI-SCREEN technology builds upon a unique optimized gene library of 33 genes associated with apoptosis, cell cycle control and stemness. After transduction with the library, all generated cell lines are screened for self-selected clonal isolates with expansion capacity consistent with immortalization. Screening a high number of immortalized cell lines identifies immortalization gene sets that are cell type specific.

Characterization of Human Thyrocyte Cell Lines





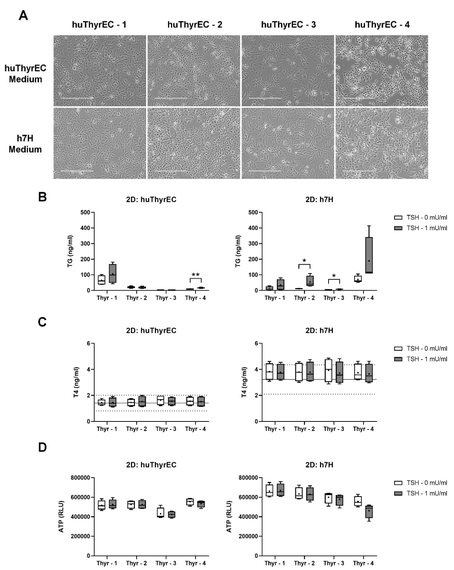
(A) Post-expansion micrographs of cell lines 1-4 in huThyrEC medium. Scale bar is 100 µM.

(B) Cumulative population doubling levels (cPDL) for cell lines 1-4. Linear regression analysis was performed from 20 days post-transduction to the final day in culture for each line. Doubling time (DT) was calculated in hours (h).

(C) KaryoStat Assay array results for cell lines 1-4. Array consisted of 18,018 copy number and 148,450 SNP probes, supporting detection of copy number variants, allelic imbalance, loss of heterozygosity, and unbalanced translocations across all 46 human chromosomes. Chromosome number (x-axis), signal intensity $\log_2$ ratios indicating copy number state (right y-axis).

(D) Integrated transgene profile for cell lines 1-4. Black box (positive) and gray box (negative).

Functional Assessment in a 2D Culture Model



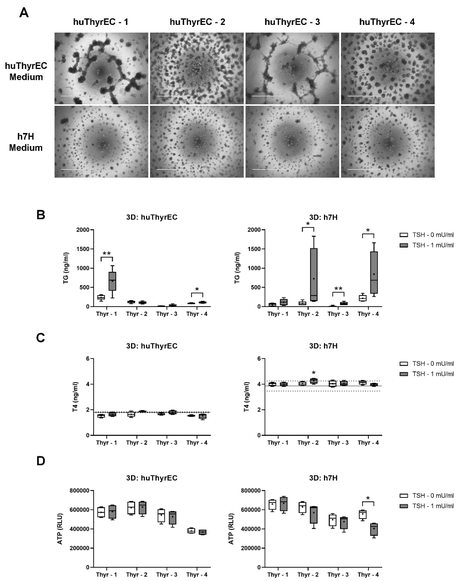
(A) Post-expansion micrographs of huThyrEC cell lines 1-4 in huThyrEC or h7H medium. Scale bar is 100 µM.

(B) Thyroglobulin (TG) production (ng/ml) in huThyrEC or h7H medium after eight days in 2D monolayer culture (n=4). Cell lines were exposed to vehicle control (TSH – 0 mU/ml) or treatment (TSH – 1 mU/ml) every two days for duration of culture. Unpaired, one-way t-tests were used to determine statistical significance (* $p < 0.05$; ** $p < 0.01$) between TSH treatment groups.

(C) Thyroxine (T4) secretion (ng/ml) in huThyrEC or h7H medium after 12 days in 2D monolayer culture (n=4). Cell lines were exposed to vehicle control (TSH – 0 mU/ml) or treatment (TSH – 1 mU/ml) every two days for duration of culture. Solid horizontal line is the mean concentration of indicated medium and dashed horizontal lines are three standard deviations from the mean. No statistically significant differences were observed.

(D) ATP values measured as relative light units (RLU) from samples in huThyrEC or h7H medium after 12 days in 2D monolayer culture (n=4). Cell lines were exposed to vehicle control (TSH – 0 mU/ml) or treatment (TSH – 1 mU/ml) every two days for duration of culture. No statistically significant differences were observed.

Functional Assessment in a 3D Culture Model



(A) Day three post-seeding micrographs of huThyrEC cell lines 1-4 in huThyrEC or h7H medium in a 3D microtissue model. Scale bar is 100 µM.

(B) Thyroglobulin (TG) production (ng/ml) in huThyrEC or h7H medium after eight days in 3D microtissue culture (n=5). Cell lines were exposed to vehicle control (TSH – 0 mU/ml) or treatment (TSH – 1 mU/ml) every two days for duration of culture. Unpaired, one-way t-tests were used to determine statistical significance (* $p < 0.05$; ** $p < 0.01$) between TSH treatment groups.

(C) Thyroxine (T4) secretion (ng/ml) in huThyrEC or h7H medium after 12 days in 3D culture (n=4). Cell lines were exposed to vehicle control (TSH – 0 mU/ml) or treatment (TSH – 1 mU/ml) every two days for duration of culture. ANOVA with post-hoc Dunnett's test was used to determine significance of T4 concentrations relative to background medium. Statistical significance above background (* $p < 0.05$).

(D) ATP values measured as relative light units (RLU) from samples in huThyrEC or h7H medium after 12 days in 3D culture (n=4). Cell lines were exposed to vehicle control (TSH – 0 mU/ml) or treatment (TSH – 1 mU/ml) every two days for duration of culture. Unpaired, two-way t-tests were used to determine statistical significance (* $p < 0.05$) between TSH treatment groups.

Summary

	2D Culture Model		3D Culture Model	
Cell Line	**huThyrEC Media**	**h7H Media**	**huThyrEC Media**	**h7H Media**
1	NS	NS	TG Production**	NS
2	NS	TG Production*	NS	TG Production* T4 Secretion*
3	NS	TG Production*	NS	TG Production**
4	TG Production**	NS	TG Production*	TG Production*

NS = No significant difference, * $p < 0.05$; ** $p < 0.01$

Conclusion

Development and characterization of four human immortalized thyrocyte cell lines identified lines exhibiting morphological and functional features of primary human thyrocytes.

- huThyrEC cells retained partial properties expected of human thyrocytes when cultured under conditions permissive to reproducing structural and hormonal cues of the native gland.
- T4 concentrations were just above background level for cell line 2. Given the concomitant increase in TSH-dependent TG in this line, cell line 2 retains features of differentiated thyrocytes that enable modest hormone synthesis.
- Novel huThyrEC cell lines are a valuable addition for *in vitro* disease modeling and toxicity testing and represent an important step toward longer term culture models that more closely represent normal human thyroid function.