Thyroid Cancer in Nebraska: How Molecular Regulators Could Impact Prevention

3rd Annual Cancer Risk and Prevention Symposium
23rd February 2024

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University of Nebraska Medical Center

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Disclosures

None

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Learning Objectives

- Appreciate the burden of thyroid cancer
- Identify components of tumor immune microenvironment promoting thyroid carcinogenesis
- Understand the role of novel molecular regulators in thyroid carcinogenesis

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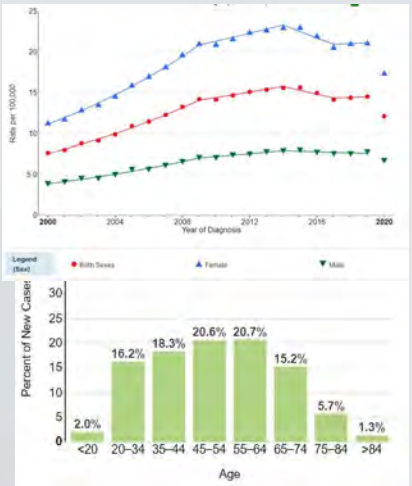
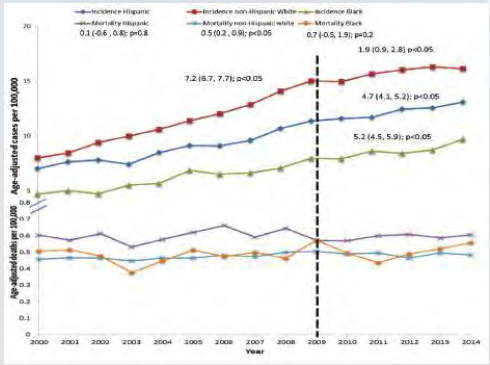
Thyroid Cancer Epidemiology

Most common endocrine cancer

Incidence x3 in 30 y → by 2030: 2nd in F

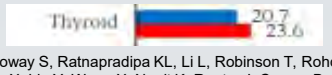


https://seer.cancer.gov/statistics-network/explorer/application



Kotwal A, Brito JP. Endo Pract 2019

Nebraska: Incidence in females per 100,000 higher than national average



Watanabe-Galloway S, Ratnapradipa KL, Li L, Robinson T, Rohde J, Luma LL, Carritt N, Zhang X, Liu Y, Wang X, Napit K, Ranta, J. Cancer Burden in Nebraska (2022). University of Nebraska Medical Center.

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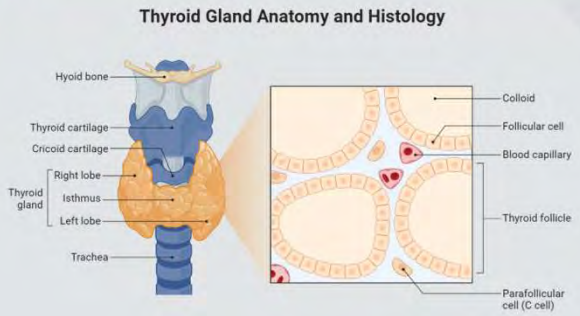
Thyroid Cancer Subtypes

- Follicular cell-derived
  - **Differentiated**
    - Papillary
    - Follicular
    - Oncocytic (Hurthle cell)
  - Poorly differentiated
  - Anaplastic
- Parafollicular cell/C cell-derived
  - Medullary



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Why Prevent and Control Thyroid Cancer?

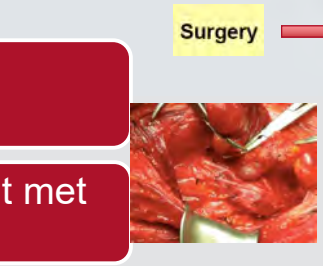
Poor QOL on par with more aggressive cancers

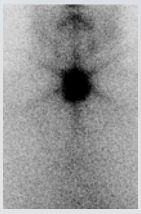
Lymph node met 60%; distant met 40%

- Multiple mets → 5 y survival 15%
- Refractory to conventional treatment → 10 y survival 10%

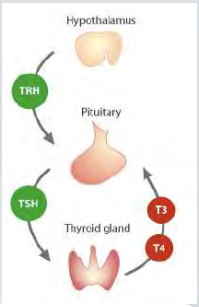
Financial Burden to patients, society

- Bankruptcy rate 9/1000 person-years >other cancers; 4.4-fold than controls





Surgery → Radioactive iodine + TSH suppression



Adverse effects of each treatment

Kotwal A, Thompson G, DeVita Cancer: Principles and Practice of Oncology 2022
Haugen B et al, Thyroid. 2016
Rahib L et al. Cancer Res. 2014
McIntyre C et al, Int J Surg. 2018
Amin MB et al, Springer. 2017

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Risk Factors for Thyroid Cancer

Ionizing radiation exposure
- 2-5 G; 2-3 decades after exposure
- 40% of nodules malignant

Family history in 1st degree relative (>2)
- 5-9 x increase

Familial adenomatous polyposis

? Thyroid autoimmunity or immune-dysregulation
- ? Elevated TSH; increased risk but better prognosis?

? Environmental exposures

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Kotwal A, Thompson G, DeVita Cancer: Principles and Practice of Oncology 2022

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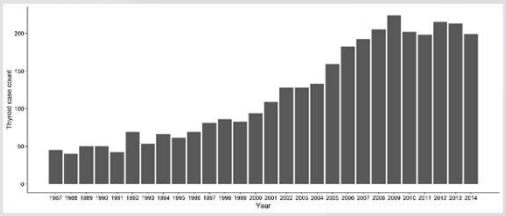
Investigating other Risk Factors for Thyroid Cancer

Whitney Goldner, MD

Variables associated with late stage (III/IV) presentation of thyroid cancer	Adjusted OR (95% CI)	P value
Urban		
Rural	**1.6 (1.04-2.4)**	0.032
Sex		
Male	Reference	
Female	0.53 (0.34-0.8)	0.005
Age at Diagnosis		
65-95 years	Reference	
45-64 years	0.56 (0.35-0.89)	0.014

Diagnosis at advanced cancer stage in rural areas of Nebraska



Agricultural exposures link with Thyroid cancer in Nebraska?

Jesse Bell, PhD

Siddhi Munde, MS

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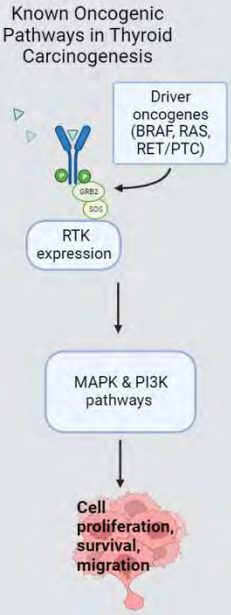
Known oncogenic pathways in Thyroid Cancer



Tyrosine Kinase inhibitors are **non-curative** and have **toxicities**

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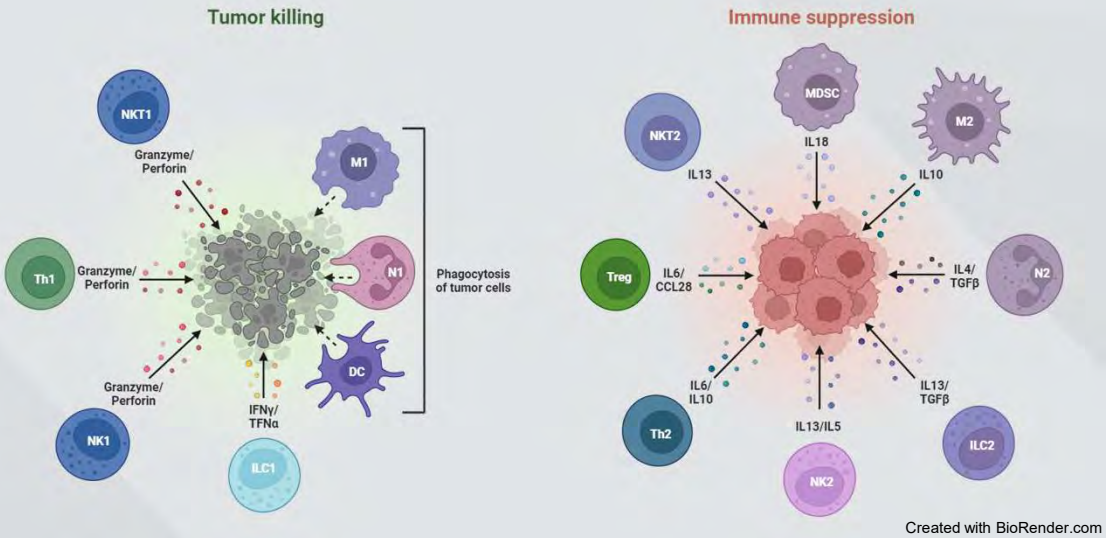
Immunoediting in Carcinogenesis



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What we know about Immunoediting in Thyroid Cancer

Lower mutation burden than others but high T-cell infiltration *(French J et al. Lancet Diab Endocrinol 2016)*

Thyroid Cancer link with Autoimmunity & Female sex

- Humans: Increased cancer risk *(Mcleod et al. J Clin Oncol. 2022)* but unclear carcinogenesis or prognosis
- Mice: Less aggressive cancer in preexisting thyroid inflammation *(Pani et al. Endocrinol. 2021)*

PD-L1 expression higher in *BRAFV600E* mutant thyroid cancer

Role of immune cells in pathological aggressiveness

- Regulatory (FOXP3+), PD1+ T cells *(French J et al. J Clin Endocrinol Metab 2012)*
- M2 macrophages *(Ryder M et al. Endocr Relat Cancer 2008; Jung KY et al. J Pathol Transl Med 2015)*
- NK cells conflicting evidence *(Gogali F et al. Thyroid 2013)*
- MDSCs *(Angell T et al. Thyroid 2016, Cunha LL et al. Clin Endocrinol (Oxf) 2013)*

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Timeline of Identifying Molecular Regulators of Thyroid Cancer

NIH National Center for Advancing Translational Sciences

MAYO CLINIC

Circulating immune phenotypes

Tumor Immune Microenvironment (TIME)

Interplay between EHD1 & Relaxin in TIME

Mabel Ryder, MD

Michael Gustafson, PhD

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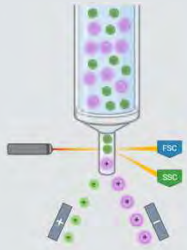
Flow cytometry on peripheral blood from n=33 Thyroid Cancer subjects

- 70% Differentiated Thyroid Cancer
- 50% with advanced such as stage IV with distant mets and high risk
- 2/3rd with lateral neck lymph node mets Follow up median 5 years



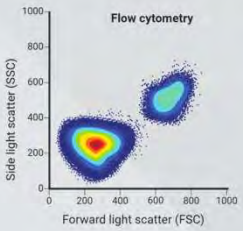


Table 1. 10-color flow cytometric protocols that enable the quantification of all major leukocyte populations.

Lyse No Wash with FlowCount Panel

Protocol	FL1	FL2	FL3	FL4	FL5	FL6	FL7	FL8	FL9	FL10
Verify	CD8	CD2	CD20	CD14	CD3	CD7	CD19	CD5	CD4	CD45
TBNK/M/G	CD15	γδTCR	CD16	CD18	CD56	CD19	CD8	CD3	CD4	CD45
Lyse Wash Panel										
Verify	CD8	CD2	CD20	CD14	CD3	CD7	CD19	CD5	CD4	CD45
T Cell-1	CCR7	CD27	CD45RO	CD25	CD3	CD62L	CD127	CD45RA	CD4	CD8
T Cell-2	CCR7	CD272	CD45RO	TIM-3	PD-1	CTLA4	CD8	CD28	CD4	CD3
B cell	IgD	CD27	CD20	CD38	CD5	IgM	CD19	CD24	CD21	CD45
Myeloid	LIN2	CD123	HLA-DR	CD11c	CD11b	CD33	CD16	CD66b	CD15	CD45
Monocytes-1	CD80	CD142	CD14	CD32	CD64	CD86		CD16	HLA-DR	CD45
Monocytes-2	B7H1	TNFR2	CD14		PD-1	CD40		CD16	HLA-DR	CD45
Granulocytes	CD66b	CD63	CD14	CD44	CD203c	CCR3	CD16	CD49d	CD15	CD45

doi:10.1371/journal.pone.0121546.t001

Gustafson MP et al. PLoS ONE. 2015

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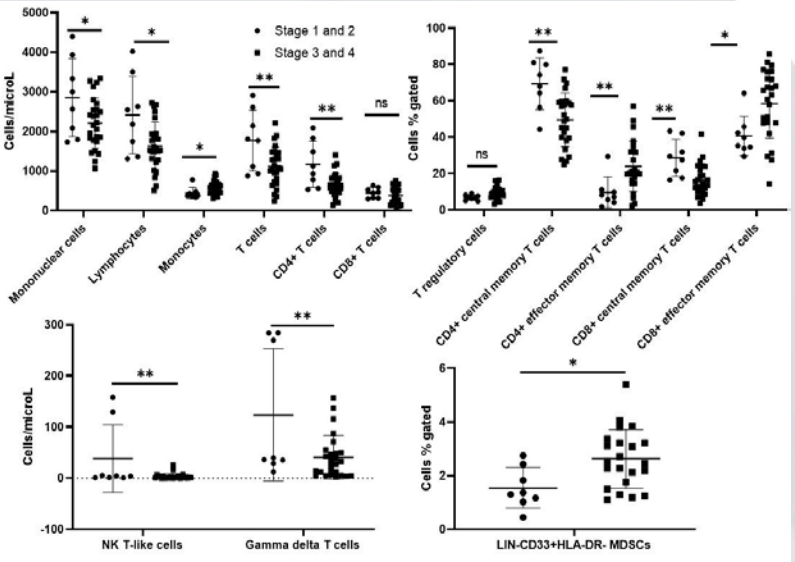
Circulating immunophenotypes are potentially prognostic in follicular cell-derived thyroid cancer

Anupam Kotwal[1,2], Michael P. Gustafson[3,4], Svetlana Bornschlegl[3], Allan B. Dietz[3,5,6], Danae Delivanis[2] and Mabel Ryder[2,7]*

[1]Division of Diabetes, Endocrinology and Metabolism, University of Nebraska Medical Center, Omaha, NE, United States, [2]Division of Endocrinology, Diabetes, Metabolism, and Nutrition, Mayo Clinic, Rochester, MN, United States, [3]Divisions of Experimental Pathology and Transfusion Medicine, Mayo Clinic, Rochester, MN, United States, [4]Division of Laboratory Medicine, Department of Laboratory Medicine and Pathology, Mayo Clinic Arizona, Phoenix, AZ, United States, [5]Department of Laboratory Medicine and Pathology, Mayo Clinic, Rochester, MN, United States, [6]Department of Immunology, Mayo Clinic, Rochester, MN, United States, [7]Division of Medical Oncology, Mayo Clinic, Rochester, MN, United States

frontiers | Frontiers in Immunology



Peripheral blood immunophenotyping via flow cytometry comparing patients with AJCC stage III/IV vs I/II thyroid cancer. *p<0.05; **p<0.01

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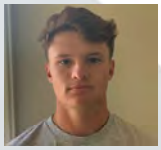
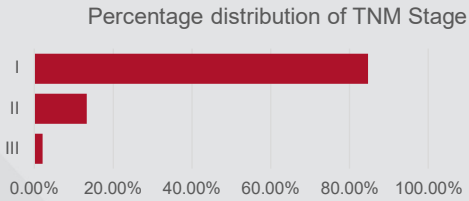
What about Thyroid Autoimmunity before Thyroid Cancer?



PJ Loucks

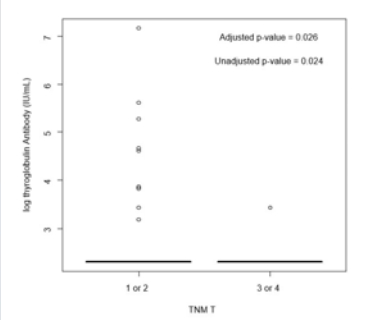
92 non-metastatic DTC adults undergoing surgical treatment with available preoperative sera



Circulating thyroid Ab	N elevated/N available (%)
TgAb >20 IU/mL	10/98 (10.20%)
TPOAb >0.3 IU/mL	34/47 (72.34%)

Higher TgAb level associated with

- **lower T stage** after adjusting for age, sex (p=0.03)
- less tumor size (p=0.04)
- lower lymph node ratio (p=0.01)



Log-transformed TgAb compared between TNM T categories 1+2 v/s 3+4

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Circulating Immune Phenotypes in Advanced Thyroid Cancer

More Suppressor Cells

- **MDSCs**
  - Immunosuppressive in sq cell, breast, lung
  - *Angell et al*: more in advanced thyroid cancer
- **Trend for Tregs**
  - Produce IL-10 to inhibit anti-tumor
  - *Need more investigation*

Less Effector Cells

- **CD4:CD8**
  - Role of CD4+ cytotoxic, helper T cells against cancer
- **Gamma-delta T cells**
  - Modulate cell proliferation, proinflammatory cytokine secretion
  - *Promote thyroid autoimmunity*
- **NK T-like cells**
  - *G et al*: Lower in metastatic colorectal Ca

Altered Memory T cells

- **Less Central Memory T cells**
  - Express CCR7
  - Sustained immune response
- **More Effector Memory T cells**
  - Lack CCR7
  - Immediate immune response
  - *Sancho et al*: Less in PDTC also

Less circulating Anti-thyroid Ab

- TPOAb
- TgAb

Angell T et al, Thyroid. 2016
French J et al, J Clin Endocrinol Metab. 2012
Gogali F et al, Thyroid. 2013
Cunha LL et al, Clin Endocrinol (Oxf). 2013
Liu H et al, Endocrine. 2016
Gharagozloo M et al, Bratisl Lek Listy. 2018
Harris NL et al, J Exp Med. 2002
Roberts AD et al, J Exp Med. 2005
Sancho M et al, J Endocrinol. 2006

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Timeline of Identifying Molecular Regulators of Thyroid Cancer

UNMC College of Medicine
NEBRASKA DEPT. OF HEALTH AND HUMAN SERVICES
GREAT PLAINS IDeA | Clinical and Translational Research

Circulating immune phenotypes

Tumor Immune Microenvironment (TIME)

Interplay between EHD1 & Relaxin in TIME

Tony Hollingsworth, PhD

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FRED & PAMELA BUFFETT CANCER CENTER

THE INTEGRATED CANCER REPOSITORY FOR CANCER RESEARCH iCaRe²

Thyroid Cancer and Tumor Collaborative Registry (TCCR)

Oleg Shats, MS for **Data curation**
Madelyn Fitch for **TMA creation and BRAF IHC**

Whitney Goldner, MD

Tissue Sciences Facility

Tissue Procurement Shared Resource

Research Tissue Bank

Benjamin Swanson, MD, PhD

Ana Yuil-Valdes, MD

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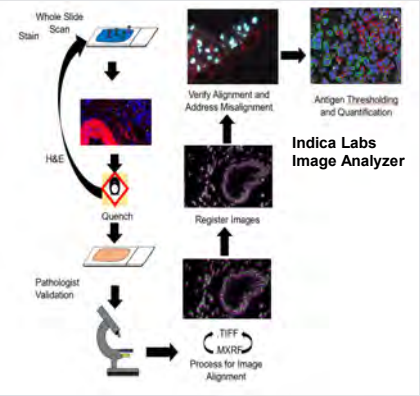
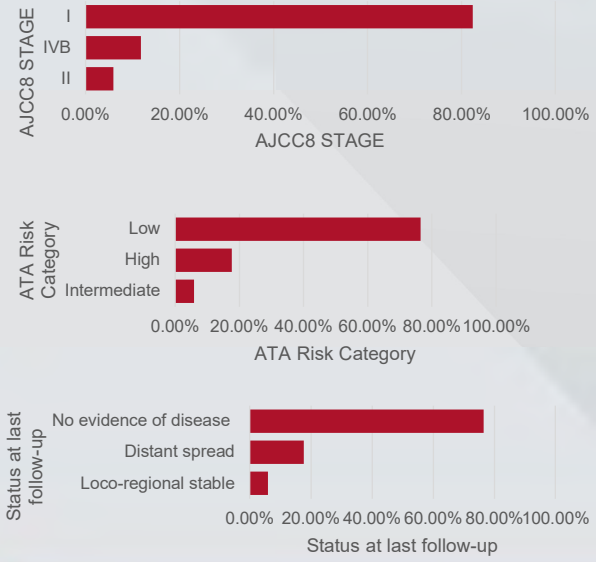
TIME comparing tumor to adjacent by multiplex immunofluorescence

- N=17 paired FFPE cancer and adjacent non-cancerous thyroidectomy tissue
  - Tonsil positive control





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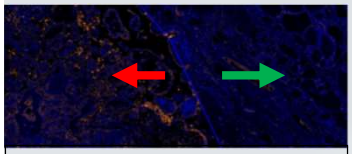
Abstract citation ID: bvac150.1764

Thyroid

RF11 | PSAT272

Suppressive Tumor Immune Microenvironment is Associated with Aggressive Differentiated Thyroid Cancer

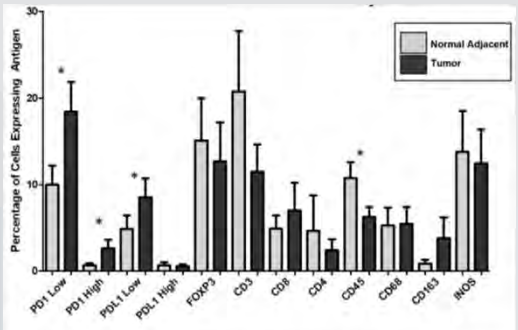
Anupam Kotwal, MD, Kemal Hajric, Undergraduate, Krysten Vance, BS, Ernesto M. Duarte, MD, MPH, Ana Yuil-Valdes, MD, Michael Hollingsworth, PhD, Apar Ganti, MD, Hamid Band, MD, PhD, Benjamin Swanson, MD, PhD, Robert Bennett, PhD, and Whitney Goldner, MD



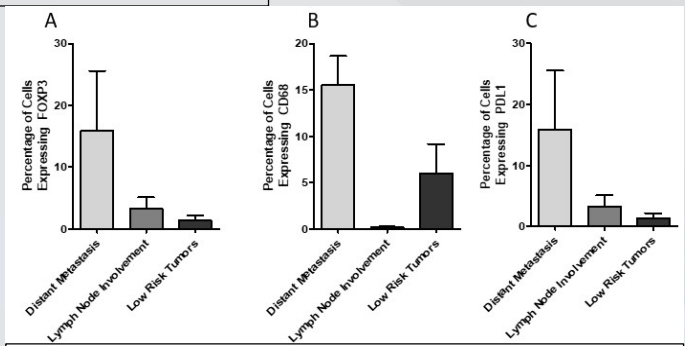
PD-1 expression in orange at the leading edge between tumor (red arrow) and adjacent thyroid (green arrow)

Kemal Hajric, BS

Krysten Vance, PhD



*Comparison of antigen expression within the tumor and adjacent thyroid tissue. *p<0.05.*



Comparison of intra-tumoral antigen expression between DTC groups with distant metastases (n=3) vs. neck lymph node involvement (n=3) vs. limited to thyroid or low risk (n=11)

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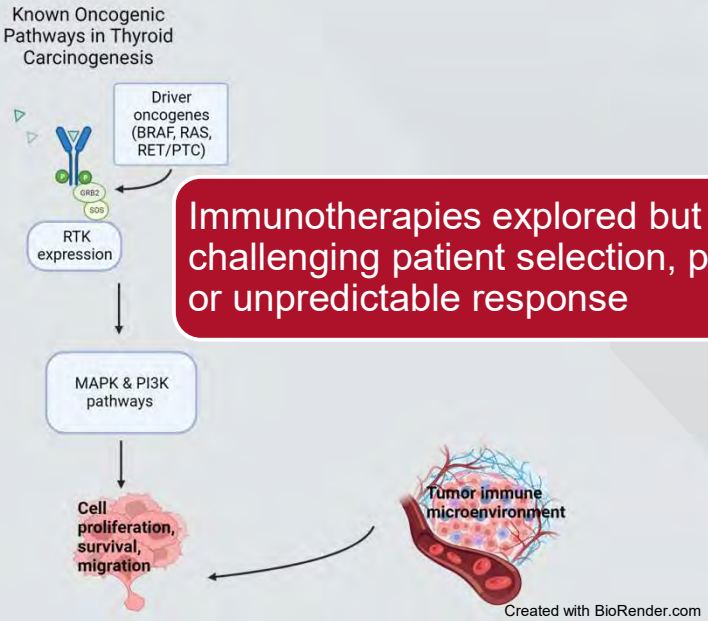
TIME influences Thyroid Carcinogenesis



Immunotherapies explored but challenging patient selection, poor or unpredictable response

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Timeline of Identifying Molecular Regulators of Thyroid Cancer

GREAT PLAINS IDeA | Clinical and Translational Research

Circulating immune phenotypes

Tumor Immune Microenvironment (TIME)

Interplay between EHD1 & Relaxin in TIME

Robert Bennett, PhD

Hamid Band, MD, PhD

Jennifer Larsen, MD

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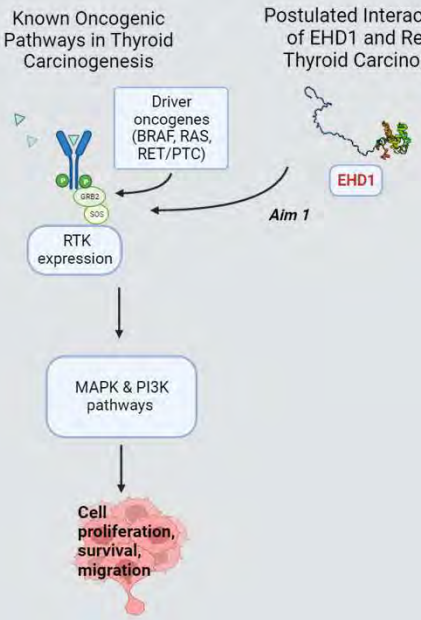
Role of EHD1 in Thyroid Carcinogenesis



EGFR pathway substrate (EPS) 15 Homology Domain-containing proteins (EHD1-4) recycle surface receptors

Tanaka y et al, Cancer Biol Ther. 2011
Iseka FM et al, The J of Immunol. 2018
Grant BD et al, Traffic. 2008

Cao WM et al, Breast Cancer Res. 2015
Hsu F et al, Acta Oncol. 2017
Liu Y et al, Oncol Lett. 2018

Wang X et al, Cell Death Dis. 2018
Wang C et al, Thorac Cacer. 2023

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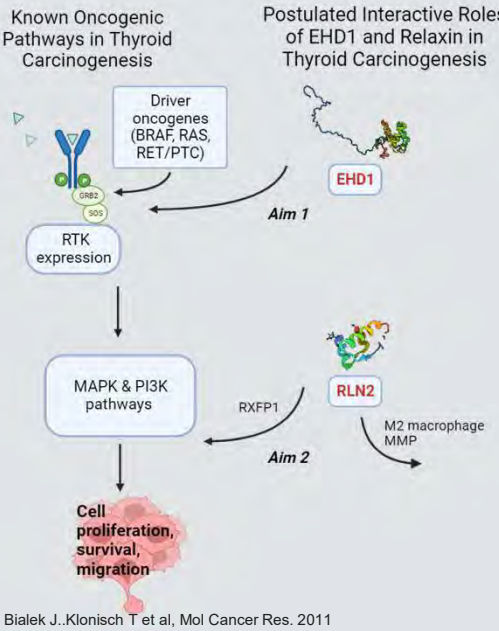
Role of Relaxin in Thyroid Carcinogenesis



Insulin-like superfamily, consists of 7 peptides

Relaxin (RLN2)

Paracrine functions of ECM remodeling, Macrophage polarization

Linked with BRAFV600E alteration in thyroid cancer

Bennett RG et al, Transl Res. 2009
Bialek J et al, Mol Cancer Res. 2011
Plunkett ER et al, J of Endocrinol. 1963
Braverman LE et al, Endocrinol. 1963

Bialek J..Klonisch T et al, Mol Cancer Res. 2011
Hombach-Klonisch S et al, The Amer J of Path. 2006
Hernandez BY et al, J of Cancer Res and Clin Oncol. 2021
Radestock Y… Hombach-Klonisch S et al, Mol Cancer Res 2010

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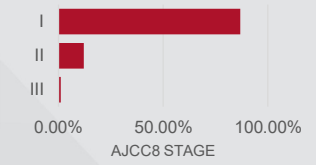
EHD1 & RLN2 expression in Thyroid Cancer TMA

Case-control study of adult humans
181 differentiated thyroid cancer (DTC)
185 benign thyroid



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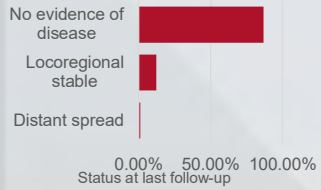

Tissue microarrays
2 cores of 2 mm per specimen
Placenta control

Antibody staining
RLN2 or EHD1
CD68 (total macrophages)
CD163 (M2 macrophages)
DAPI (nucleus)

Analysis
Pixels/area experimental slide – isotype-matched IgG slide
Mean pixels/area or EHD1 histoscore (staining intensity x % cells stained) compared by t-test assuming unequal variances

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Thyroid Cancer Translational Poster

EPS15 HOMOLOGY DOMAIN 1 PROTEIN: A NOVEL BIOMARKER FOR DIFFERENTIATED THYROID CANCER

Anupam Kotwal*, Bhopal Mohapatra, Sukanya Chakraborty, Maddie Fitch, Matthew Storck, Nicholas Whiteman, Joshua Nguyen, Cheng Zheng, Ana Yuil-Valdes, Benjamin Swanson, Oleg Shats, Robert Bennett, Hamid Band, Whitney Goldner

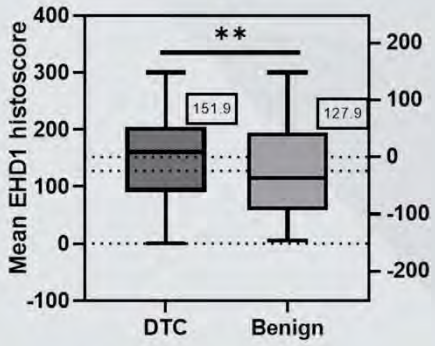
Bhopal Mohapatra, PhD

Cases (n=181) vs Controls (n=185)

Higher EHD1 histoscore

- Difference of means 23.8 (95% CI 7.3 to 40.3; p=0.005)
- Persisted after adjusting for age and sex
  Difference of means 24 (95% CI 10.8 to 44.5; p=0.001)

Not associated with
Sex, age
AJCC stage, lymph node status or ATA risk category (limited sample)



*Comparison between cases (DTC without distant metastases) and controls (benign thyroid) in terms of EHD1 histoscore. **p<0.01*

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Abstract citation ID: bvad114.1996

Thyroid

SAT524

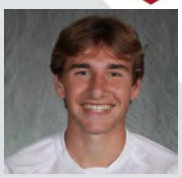
Relaxin: A Novel Marker for Pro-Tumoral Thyroid Cancer Microenvironment?

***Anupam Kotwal, MD**, Robert G. Bennett, Benjamin Swanson, Whitney Goldner, Ana Yuil-Valdes, Nicholas Whiteman, and Oleg Shats*

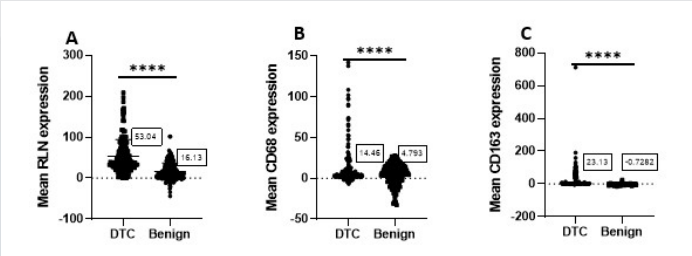
University of Nebraska Medical Center, Omaha, NE, USA

Cases (n=181) vs Controls (n=185)

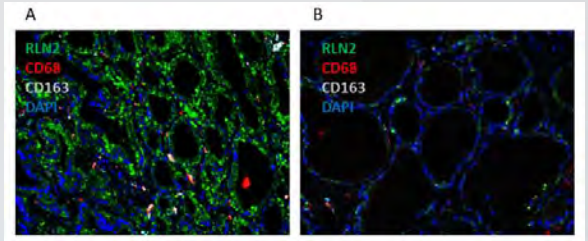
- **Higher RLN2**, CD68 (macrophage) and CD163 (M2 macrophage) p<0.0001
- Not associated with sex, age, AJCC stage, lymph node status or ATA risk category (limited sample)



Nicholas Whiteman, BS



*Comparison between cases (DTC without distant metastases) and controls (benign thyroid) in terms of RLN2 (A), CD68 (B) and CD163 (C) expression in pixels/area. ****p<0.0001.*



Immunofluorescence image of experimental DTC core (A) and non-cancerous thyroid (B) illustrating expression of RLN2, CD68, CD163, and DAPI (nucleus).

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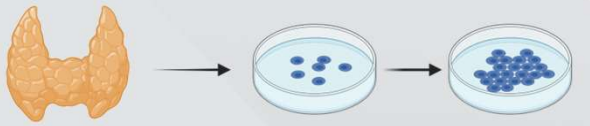
Thyroid cell lines



Created with BioRender.com

Cell Line	Type	Sex	Mutation (s)	Thyroid-specific genes
N-Thy	Noncancer	F	None	None
BCPAP	Papillary	F	BRAF-V600E RET/PTC1 p53	High PAX8 and TTF1
KTC-1	Papillary	M	BRAF-V599E	High PAX8 and TTF1
TPC-1	Papillary	F	RET/PTC1 HRAS	High PAX8 but absent TTF1
FTC133	Follicular	M		Low PAX8 but high TTF1
Hth7	Anaplastic	F	BRAF-WT	Low PAX8 and TTF1
C643	Anaplastic	M	BRAF-WT HRAS-G13R RAF1	Low PAX8 and TTF1

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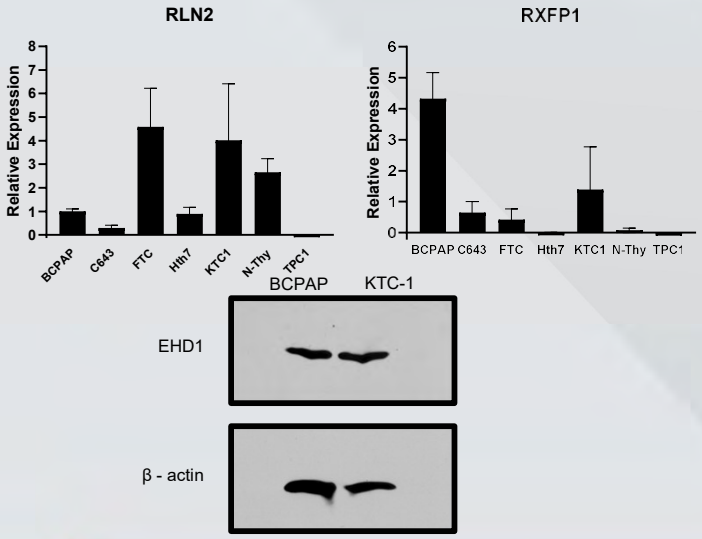
EHD1 & RLN2 expression in Thyroid Cancer cell lines – preliminary

Compare by oncogenic drivers

- immortalized cells lose differentiation

Transwell migration assay; co-culture immune cells

EHD1 KO to study impact on RLN2 and vice versa → impact on cell characteristics



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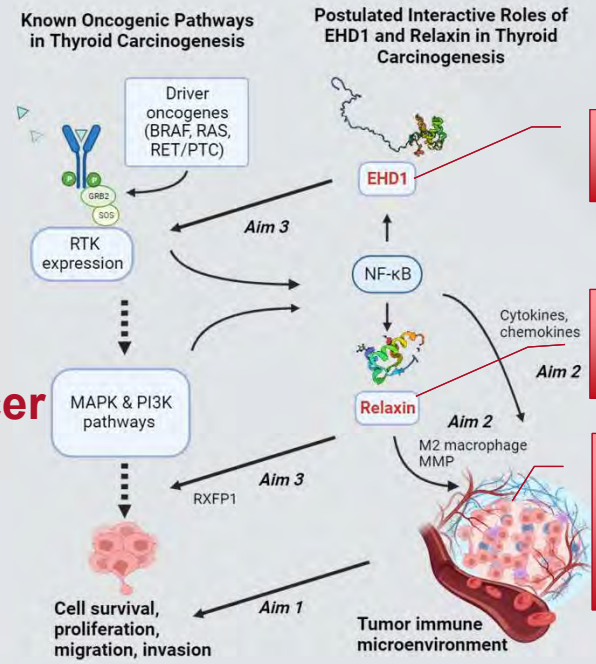
Interplay between EHD1 & Relaxin via NF-kB:

Impact on Cancer Risk and Prevention?



Druggable ATPase
RTKs driven by BRAF mutation

Timing in carcinogenesis
Modulation for prevention
Prognostic significance

Downregulate suppressors (M2, Treg, MDSC)
Upregulate effectors (CD8, NK, gamma-delta)

Burston HE et al, The Journal of Clinical Investigation. 2021
Wang C et al, Thorac Cacer. 2023

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Conclusions and Future Directions: Thyroid Cancer Risk & Prevention

- Interface of molecular regulators with immunoediting
- Influence of environmental exposures
  - Early diagnosis and risk prediction
  - Targeted strategies before cancer spread
  - Translation to other malignancies

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Thanks!

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