# Sidney H. Ingbar Lecture, ATA 10/17/2013: PAX8-PPARy Fusion Protein in thyroid carcinoma

Ronald J. Koenig, MD, PhD
Division of Metabolism, Endocrinology &
Diabetes

University of Michigan

#### Learning Objectives

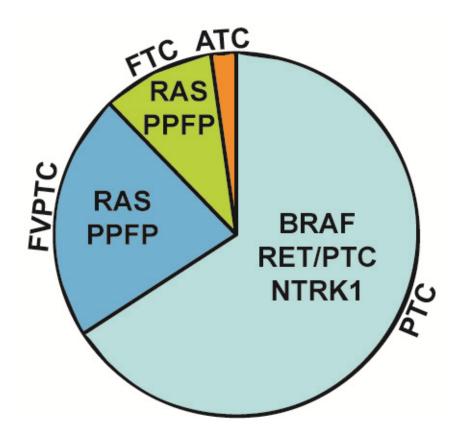
- 1. Know what the PAX8-PPARγ Fusion Protein is.
- 2. Know what types of thyroid cancer are associated with the PAX8-PPAR<sub>γ</sub> Fusion Protein.
- 3. Know about a clinical trial specifically designed for patients with PAX8-PPARγ Fusion Protein thyroid cancer.
- 4. Know the normal function of PPAR<sub>γ</sub>.
- 5 Know about the Wnt pathway.

(No disclosures)

#### Outline of today's presentation

- Background
- PPFP carcinoma patient samples
- Mouse model of PPFP thyroid carcinoma
- PPFP cell lines
- Summary and conclusions

### Gene mutations underlie thyroid carcinoma



Vu-Phan D & Koenig RJ. Mol. Cell. Endocrinol., in press (review)

#### Frequent Occurrence of Cytogenetic Abnormalities in Sporadic Nonmedullary Thyroid Carcinoma

Robert B. Jenkins, MD, PhD,\* Ian D. Hay, MB, PhD,†
John F. Herath, MS,\* Cloann G. Schultz, BS,\* Jack L. Spurbeck, BS,\*
Clive S. Grant, MD,‡ John R. Goellner, MD,§
and Gordon W. Dewald, PhD\*

Cytogenetic studies may provide important clues to the molecular pathogenesis of thyroid neoplasia. Thus, the authors attempted cytogenetic studies on 12 thyroid carcinomas: seven papillary, three follicular, and two anaplastic. Successful cytogenetic results were obtained on all 12 tumors; nine (75%) had one or more chromosomally abnormal clones. Four of the papillary carcinomas had a simple clonal karyotype, and three had no apparent chromosome abnormality. All four abnormal papillary tumors contained an anomaly of a chromosome 10q arm. In one instance, an inv(10)(q11.2q21.2) was observed in a Grade 2 papillary carcinoma as the sole acquired abnormality. In another case, an inversion or insertion involving 10q21.2 was found in a Grade 1 papillary tumor. The karyotype of a third tumor, a Grade 1 papillary carcinoma, was 46,XX,der(5)t(5;10)(p15.3;q11),der(9)t(9;?)(q11;?). A fourth abnormal papillary carcinoma, a Grade 1 tumor, had a t(6;10)(q21;q26.1) as the sole abnormality. Each of the five follicular or anaplastic carcinomas had a complex clonal karyotype. The three follicular carcinomas contained an abnormality of 3p25-p21, along with several other chromosome abnormalities. Cancer 66:1213-1220, 1990.

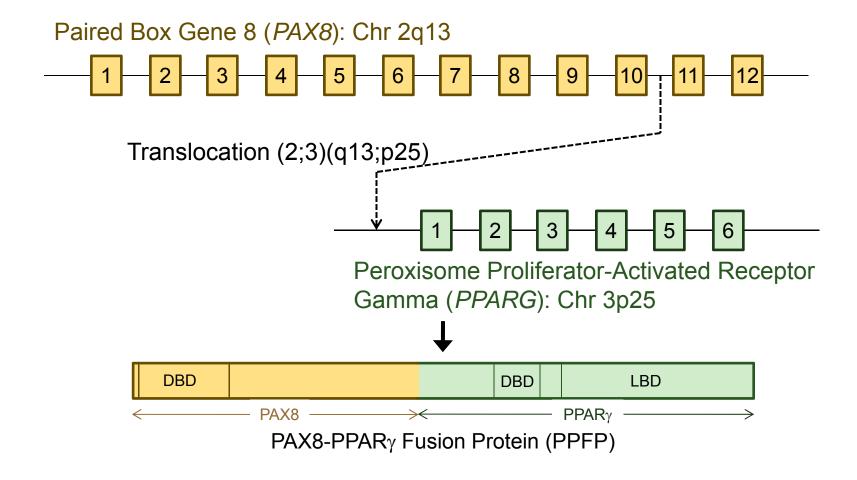
## PAX8-PPARγ1 Fusion Oncogen in Human Thyroid Carcinoma

Todd G. Kroll, 1\* Pasha Sarraf, 2 Lorenza Pecciarini, 1
Chang-Jie Chen, 1 Elisabetta Mueller, 2 Bruce M. Spiegelman, 2
Jonathan A. Fletcher 1,2,3\*

Chromosomal translocations that encode fusion oncoproteins have been observed consistently in leukemias/lymphomas and sarcomas but not in carcinomas, the most common human cancers. Here, we report that t(2;3)(q13;p25), a translocation identified in a subset of human thyroid follicular carcinomas, results in fusion of the DNA binding domains of the thyroid transcription factor PAX8 to domains A to F of the peroxisome proliferator—activated receptor (PPAR)  $\gamma1$ . PAX8-PPAR $\gamma1$  mRNA and protein were detected in 5 of 8 thyroid follicular carcinomas but not in 20 follicular adenomas, 10 papillary carcinomas, or 10 multinodular hyperplasias. PAX8-PPAR $\gamma1$  inhibited thiazolidinedione-induced transactivation by PPAR $\gamma1$  in a dominant negative manner. The experiments demonstrate an oncogenic role for PPAR $\gamma$  and suggest that PAX8-PPAR $\gamma1$  may be useful in the diagnosis and treatment of thyroid carcinoma.

www.sciencemag.org SCIENCE VOL 289 25 AUGUST 2000

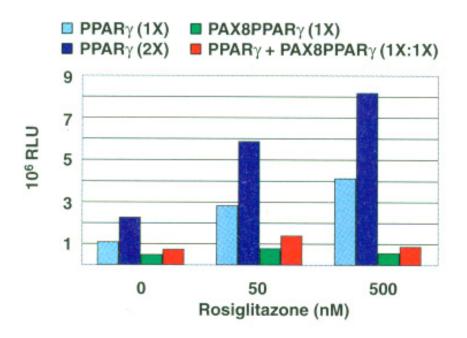
#### PPFP is generated by a chromosomal translocation



#### Functions of PAX8 and PPARy.

- PAX8 is a transcription factor that is essential for thyroid development and that drives the expression of many thyroid-specific genes.
- PPAR
   γ is the master regulator of adipogenesis, among other functions.
  - PPARγ is expressed at very low levels in normal thyroid and has no known function in that organ.

#### PAX8-PPAR $\gamma$ has dominant negative activity over PPAR $\gamma$



(transfections in U2OS osteosarcoma cells)

Kroll TG, et al. Science 289:1357-60, 2000

#### PPARγ, a putative tumor suppressor

- Several mouse models of cancer are exacerbated by a single allele deletion of *Pparg*, including a model of thyroid cancer that is unrelated to PPFP (Kato, *et al.* Oncogene 25:2736, 2006).
- Hypothesis: PPFP is oncogenic because it inhibits the tumor suppressor activity of PPARγ.

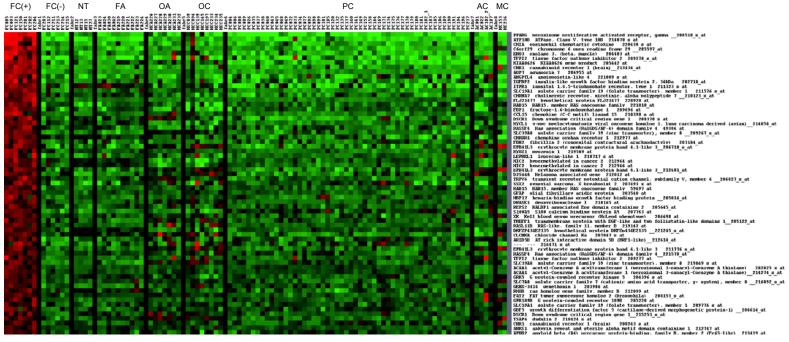
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# Gene expression signature of PPFP follicular thyroid cancers

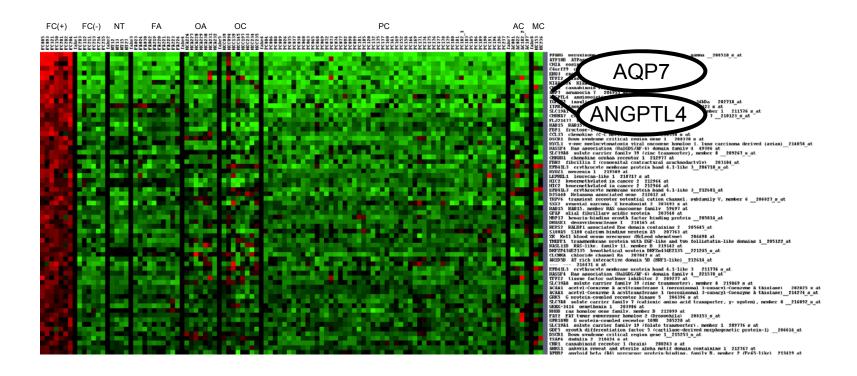


Thomas Giordano, MD, PhD



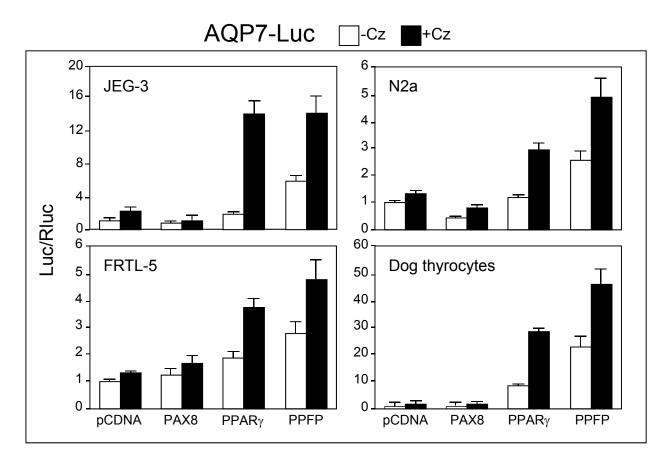
Giordano TG, et al. Clin Cancer Res. 12:1983-93, 2006

### Gene expression signature of PPFP follicular thyroid cancers.



Giordano TG, et al. Clin Cancer Res. 12:1983-93, 2006.

### PPFP has PPARγ-like activity on the AQP7 promoter.



Giordano TG, et al. Clin Cancer Res. 12:1983-93, 2006.

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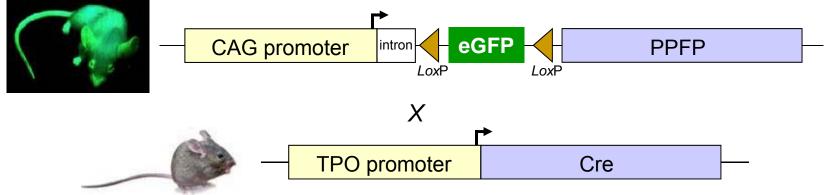
#### Thyroidal expression of PPFP in transgenic mice



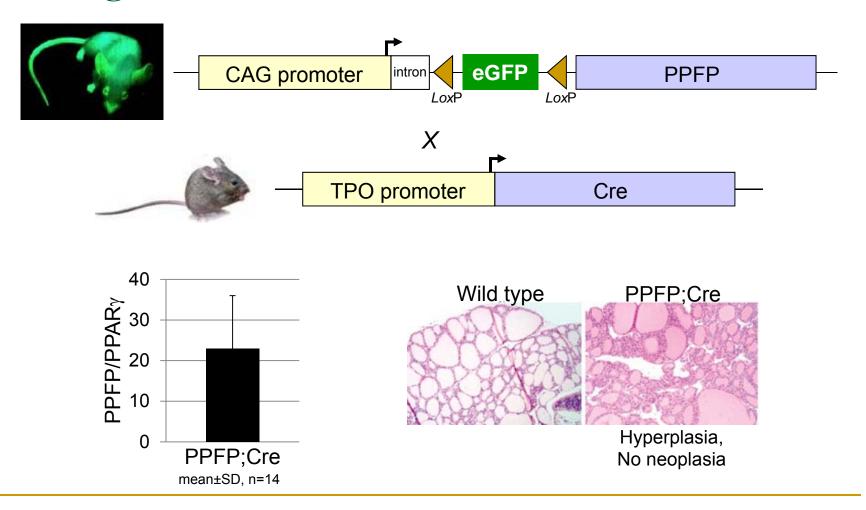


Ericka Diallo-Krou

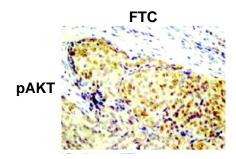
Melissa Dobson, PhD



## Thyroidal expression of PPFP in transgenic mice



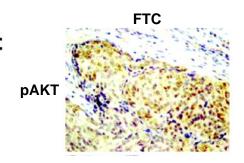
#### Highly Prevalent Genetic Alterations in Receptor Tyrosine Kinases and Phosphatidylinositol 3-Kinase/Akt and Mitogen-Activated Protein Kinase Pathways in Anaplastic and Follicular Thyroid Cancers



Zhi Liu, Peng Hou, Meiju Ji, Haixia Guan, Kimberly Studeman, Kirk Jensen, Vasily Vasko, Adel K. El-Naggar, and Mingzhao Xing

J Clin Endocrinol Metab 93: 3106-3116, 2008

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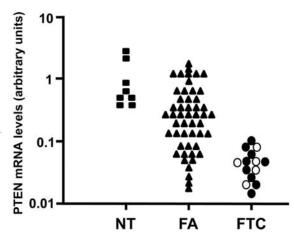
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#### Molecular Differences Between Human Thyroid Follicular Adenoma and Carcinoma Revealed by Analysis of a Murine Model of Thyroid Cancer

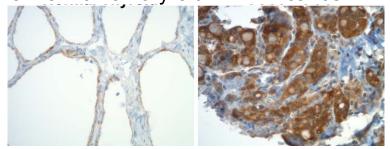
Marialuisa Sponziello, Elisa Lavarone, Enrico Pegolo, Carla Di Loreto, Cinzia Puppin, Marika A. Russo, Rocco Bruno, Sebastiano Filetti, Cosimo Durante, Diego Russo, Antonio Di Cristofano, and Giuseppe Damante

Endocrinology 154: 3043-3053, 2013



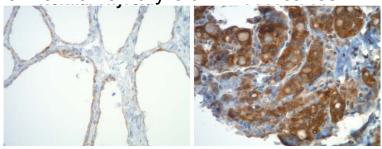
# PPFP carcinomas have increased phosphoAKT

pAKT(S473) Immunohistochemistry Normal human thyroid PPFP cancer



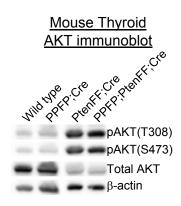
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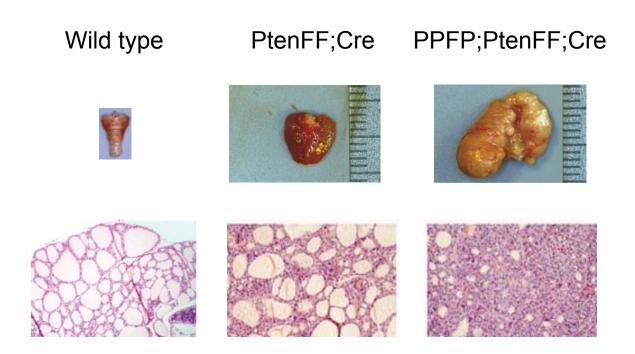


Combined PPFP expression with thyroid-specific *Pten* deletion: <u>PPFP;PtenFF;Cre</u>

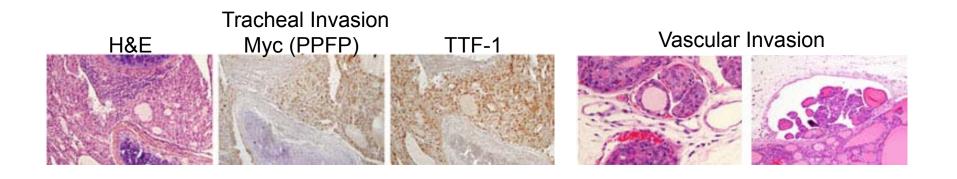
Control mice Pten deletion: PtenFF;Cre.

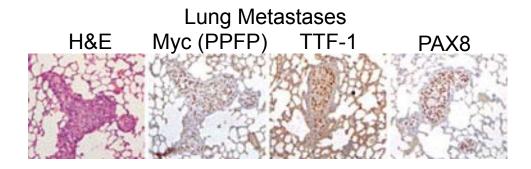


#### Mouse thyroid glands



# PPFP;PtenFF;Cre mice develop metastatic thyroid carcinoma





(PtenFF; Cre mice have benign thyroid hyperplasia with no neoplasia).

# Pioglitazone therapy of PPFP;PtenFF;Cre thyroid cancer

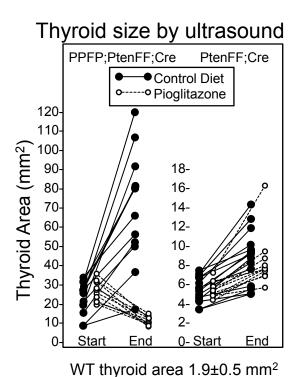
# Thyroid size by ultrasound PPFP;PtenFF;Cre Control Diet Control Diet Preserved and the proof of the preserved and the proof of the preserved and the pre

WT thyroid area 1.9±0.5 mm<sup>2</sup>

0-Start

End

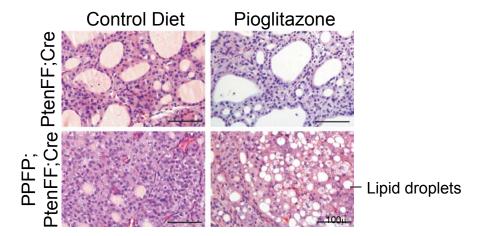
# Pioglitazone therapy of PPFP;PtenFF;Cre thyroid cancer.

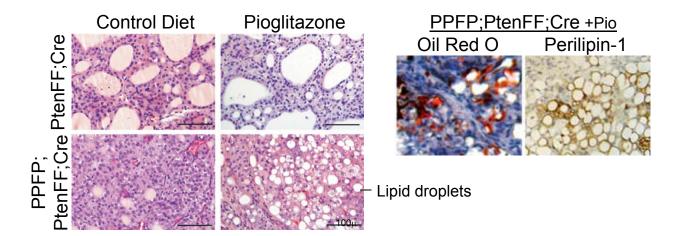


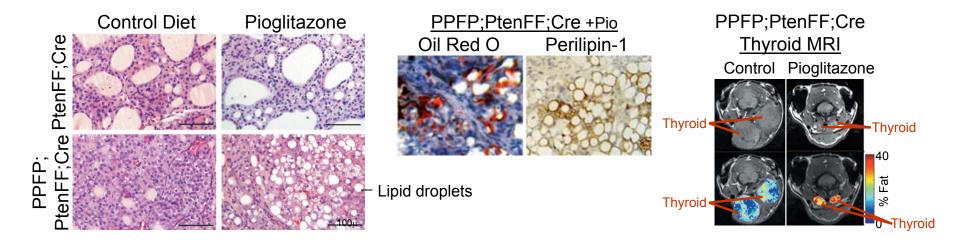
Pioglitazone prevents local invasion and metastases in PPFP;PtenFF;Cre mice

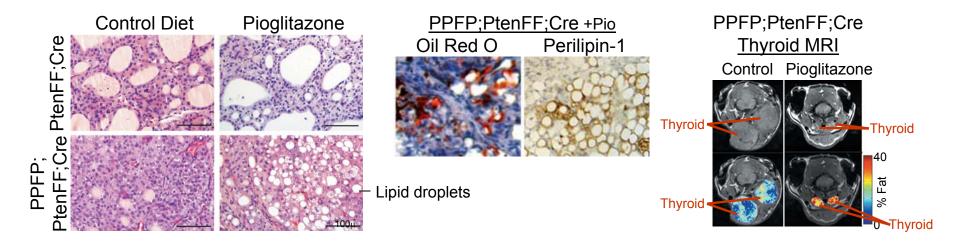
	N	Local invasion	Vascular invasion	Distant mets
Control diet	12	11	5	7
Pioglitazone	10	1	0	0
Р		0.0002	0.03	0.005

(Neither invasion nor metastases were found in any PtenFF;Cre mice)

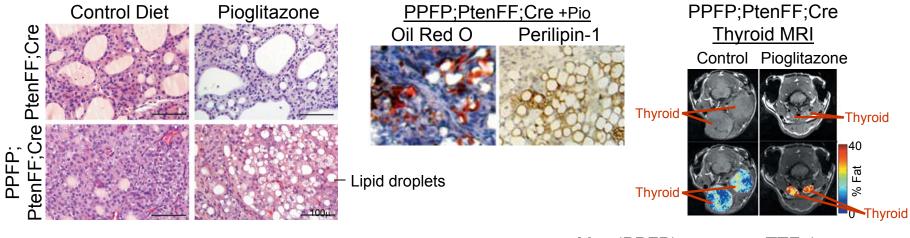




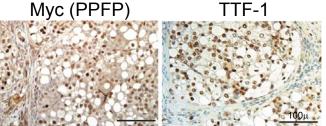


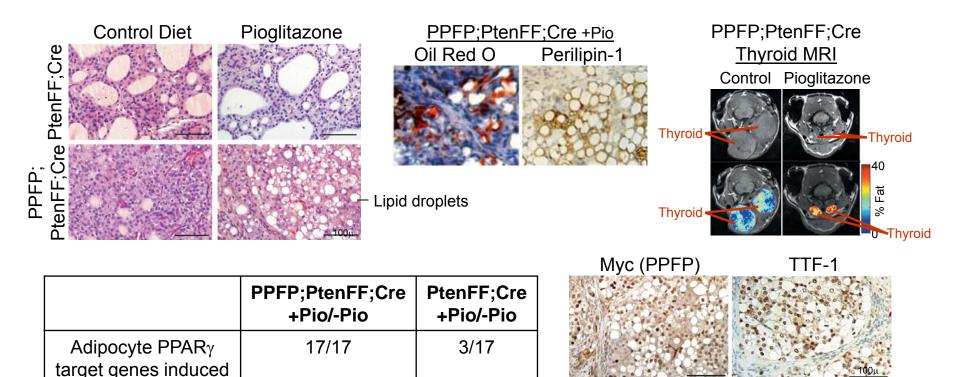


	PPFP;PtenFF;Cre +Pio/-Pio	PtenFF;Cre +Pio/-Pio
Adipocyte PPARγ target genes induced	17/17	3/17

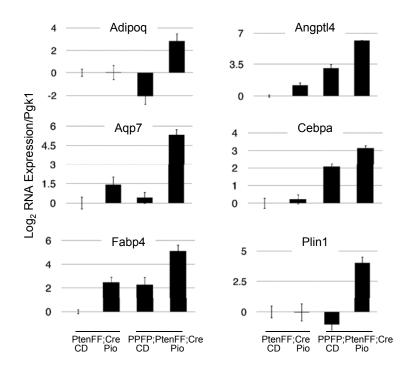


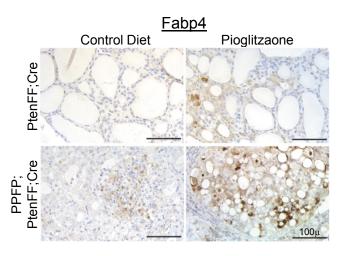
	PPFP;PtenFF;Cre +Pio/-Pio	PtenFF;Cre +Pio/-Pio
Adipocyte PPARγ target genes induced	17/17	3/17





**Hypothesis**: The therapeutic activity of pioglitazone in PPFP carcinomas is due to differentiation into adipocyte-like cells.





#### Ratios of gene expression by genotype and Pio treatment

	PtenFF;Cre	PPFP;PtenFF;Cre /	PPFP;PtenFF;Cre /	PPFP;PtenFF;Cre	
GENE	Pio/CD	PtenFF;Cre (CD)	PtenFF;Cre (Pio)	Pio/CD	
Adipoq	1.48	0.48	7.4	22.6	
Agpat2	1.08	0.82	3.1	4.0	
Angptl4	2.40	13.63	30.5	5.4	
Aqp7	3.03	1.30	11.4	28.6	
Cd36	3.93	3.54	9.7	10.7	
Cebpa	1.14	3.86	7.1	2.1	
Cidec	1.77	0.27	7.7	51.7	
Dgat1	1.38	1.53	2.0	1.8	
Fabp4	6.66	12.95	7.5	3.8	
Fasn	0.72	0.87	4.0	3.3	
Glut4	1.54	0.42	3.8	13.8	
Gpd1	1.17	4.69	9.9	2.5	
Lipe	1.37	0.86	3.5	5.6	
Lpl	1.50	0.67	5.1	11.5	
Pck1	2.55	1.29	7.8	15.3	
Pdk4	1.32	0.96	3.6	5.0	
Plin1	1.28	0.61	12.8	26.9	
P<.05 Newman-Keuls test; Pio or PPFP induces gene					
P<.05 Newman-Keuls test; Pio or PPFP represses gene					

# NCT01655719: Pioglitazone therapy of PPFP thyroid carcinomas

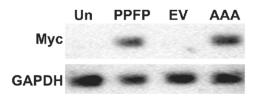
- Multi-site clinical trial.
- Local or metastatic disease not amenable to therapy with surgery or RAI.
- We screen archival tumor blocks from patients with FVPTC or FTC for the PAX8-PPAR<sub>γ</sub> translocation.
- Pioglitazone has very low toxicity; please consider referring patients to our trial!

#### Outline of today's presentation

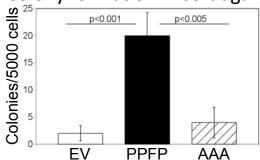
- Background
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# PPFP increases cell invasiveness and anchorage-independent growth.

PCCL3 thyroid cells stably expressing PPFP or DBD mutant AAA

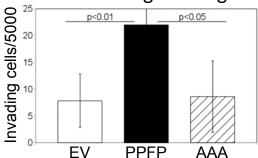


Colony formation in soft agar



Invasion through Matrigel

Dang Vu-Phan, PhD



Vu-Phan D, et al. Endocr Relat Cancer. 20:725-39, 2013.

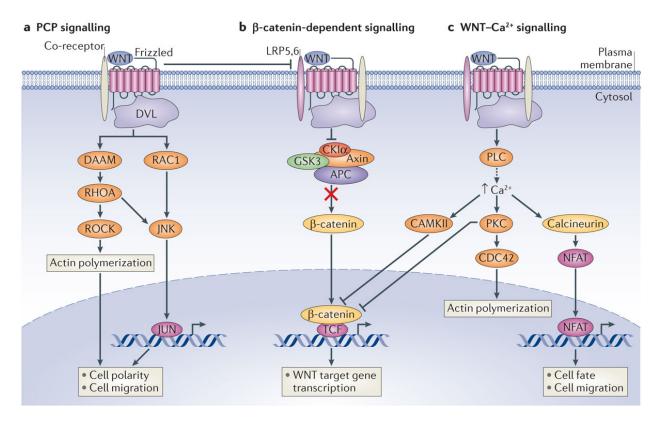
### The Connectivity Map (CMAP)

CMAP is a collection of genome-wide transcriptional expression data from cultured human cells treated with bioactive small molecules, combined with pattern-matching algorithms that together enable the discovery of functional connections between drugs, genes and diseases.

#### CMAP connects PPFP with Wnt

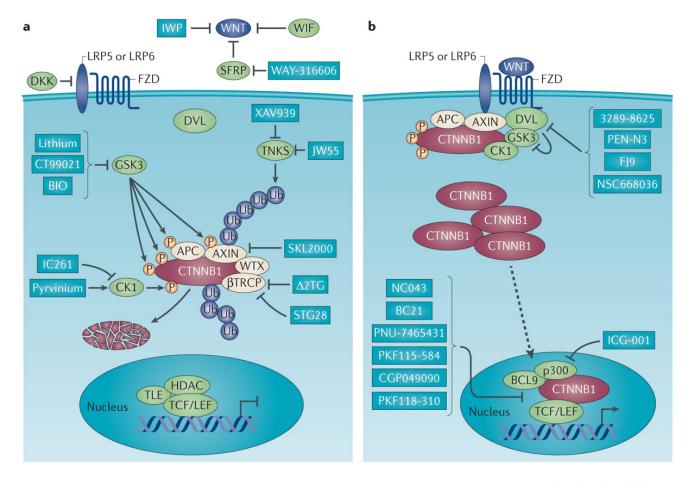
- The PPFP gene signature was analyzed in CMAP.
- 70 bioactive small molecules elicited gene expression changes that were enriched for PPFP profile genes.
- 16 of those 70 have been reported to be Wnt pathway modulators.

### Wnt signaling overview



Niehrs C. Nature Reviews Molecular Cell Biology (2012) 13:767.

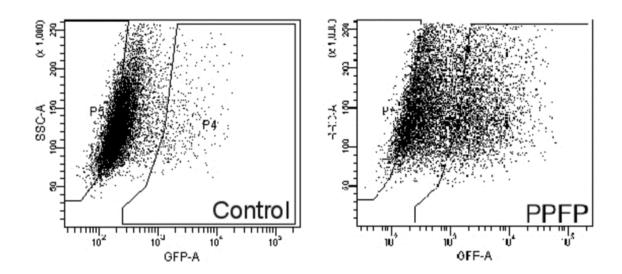
### Wnt/Tcf pathway



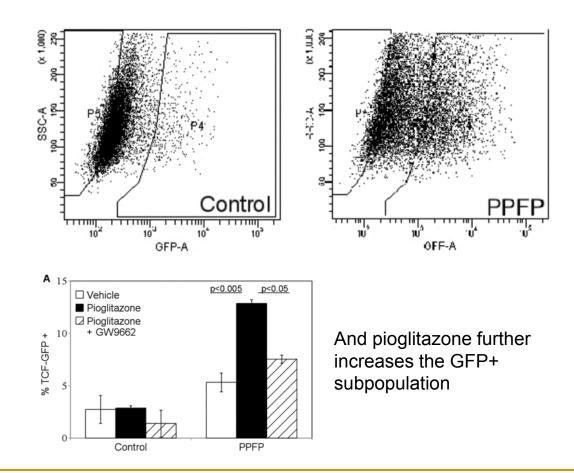
Nature Reviews | Cancer

Anastas JN and Moon RT. Nat Rev Cancer. 13:11-26, 2013

### PPFP induces a subpopulation of Wnt/TCF-activated cells

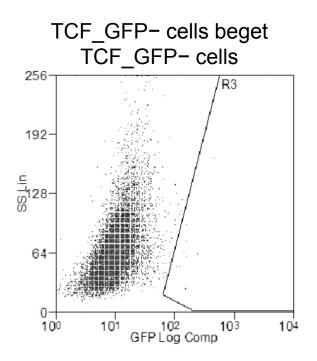


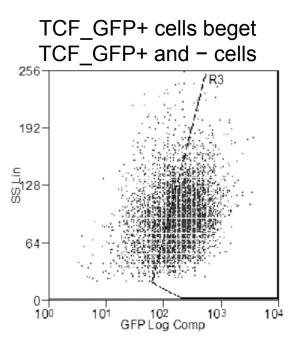
### PPFP induces a subpopulation of Wnt/TCF-activated cells



Vu-Phan D, et al. Endocr Relat Cancer. 20:725-39, 2013

# TCF\_GFP+ and TCF\_GFP- cells are hierarchically organized

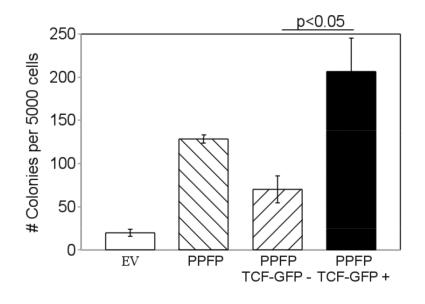


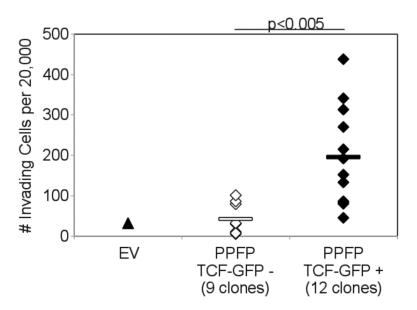


TCF\_GFP- (*left*) and TCF\_GFP+ (*right*) cells were sorted by FACS, expanded in culture, and reanalyzed for GFP status.

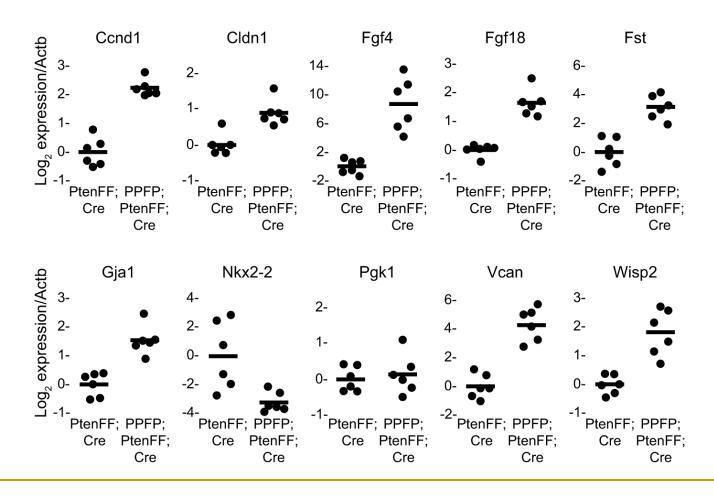
Vu-Phan D, et al. Endocr Relat Cancer. 20:725-39, 2013

## TCF\_GFP+ cells are enriched in colony formation and invasiveness





### Wnt/TCF-responsive genes are induced in a mouse model of PPFP thyroid carcinoma



Vu-Phan D, et al. Endocr Relat Cancer. 20:725-39, 2013

#### Summary and conclusions.1

- PPFP is found in FTCs and FVPTCs.
- PPFP can antagonize PPARγ or can be PPARγ-like, depending on context.
- In a mouse model of PPFP thyroid cancer, the PPARγ agonist pioglitazone is highly therapeutic and is pro-adipogenic.
- We have begun a clinical trial of pioglitazone in patients with PPFP thyroid carcinomas.

#### Summary and conclusions.2

- PPFP expression enhances the ability of PCCL3 thyroid cells to invade through Matrigel and to have anchorage independent growth.
- These actions require an intact PPARγ DBD within PPFP.

#### Summary and conclusions.3

- PPFP induces a subpopulation of Wnt/TCF activated cells.
- The PPFP-induced TCF+ population is enriched in colony forming and invading cells.
- The PPFP cells are hierarchically organized by TCF status. Based upon this, we hypothesize that the TCF+ fraction harbors PPFP thyroid cancer stem cells.