Thyroid Cancer and Genetic Differentiation

Terrence P McGarty
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Papillary Thyroid Cancer is the most common of thyroid cancers. However, like so many cancers, there are a multiplicity of sub-types and histologically determining them can be difficult. Genetically determining them should conceptually be more dispositive. We examine some recent work in this area.

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tmcgarty@telmarc.com.

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1 INTRODUCTION

Papillary thyroid cancer (PTC) is one of the generic types of thyroid cancer[1]. The others are follicular, medullary and anaplastic. Papillary thyroid cancers generally are identified by the nuclear characteristics of the cells including such things as notched, clear, and roughed[2]. General papillary thyroid cancer or what is called classical papillary thyroid cancer, CPTC, is characterize by papilla type structures and cells having the general characteristics we just mentioned. Tall cell PTC is a more aggressive type where the cell has an elongated appearance. The follicular variant of PTC, FVPTC, is the least aggressive and when encapsulated and small in size, less than 1 cm, is often considered a quasi-benign neoplasia. We have examined these types previously but some recent results are of interest regarding the genomic differences between the two.

1.1 THE ISSUE

The main issue we are exploring is the nexus between morphological shape and genetic expression. Of particular interest is these differences in papillary thyroid cancer (PTC) and its variants. More fundamentally, the question may be; what come first, the chicken or the egg. Here the chicken is the morphological shape histologically speaking and the egg is the genetic uniqueness profile.

That is, if we can determine, if you will, by say a cluster analysis and pattern recognition of differing pathological states of papillary thyroid cancers. Namely by selecting some set of genes and then examining the lesion for these genes can we delineate a set of differing PTCs comparable to what the histologist sees. One should remember that the histological categories we see today have progressed over the past century in a complex and sophisticated manner. There then is no reason to assume that such a progression is now definitive. Thus what we examine here is driven by a recent analysis.

In a recent paper by Chakladar et al, the authors note:

Papillary thyroid carcinoma (PTC) variants exhibit different prognosis, but critical characteristics of PTC variants that contribute to differences in pathogenesis are not well-known. This study aims to characterize dysregulated immune-associated and cancer-associated genes in three PTC subtypes to explore how the interplay between cancer and immune processes causes differential prognosis. RNA-sequencing data from The Cancer Genome Atlas (TCGA) were used to identify dysregulated genes in each variant[3]*. The dysregulation profiles of the*

[1] https://www.researchgate.net/publication/331935614_Thyroid_Cancer_Seek_and_Ye_Shall_Find

[2] https://www.researchgate.net/publication/334429457_miRNAs_Genes_and_Cancer_Cytology

[3] See the NCI portal as follows: https://portal.gdc.cancer.gov/exploration?filters=%7B%22op%22%3A%22and%22%2C%22content%22%3A%5B%7B%22op%22%3A%22in%22%2C%22content%22%3A%7B%22field%22%3A%22cases.primary_site%22%2C%22value%22%3A%5B%22Thyroid%20gland%22%5D%7D%7D%5D%7D also see the NCI TCGA at https://www.cancer.gov/about-nci/organization/ccg/research/structural-genomics/tcga

subtypes were compared using functional pathways clustering and correlations to relevant clinical variables, genomic alterations, and microRNA regulation.

We discovered that the dysregulation profiles of classical PTC (CPTC) and the tall cell variant (TCPTC) are similar and are distinct from that of the follicular variant (FVPTC). However, unique cancer or immune-associated genes are associated with clinical variables for each subtype.

Cancer-related genes

(i) MUC1, FN1, and S100-family members were the most clinically relevant in CPTC,

(ii) APLN and IL16, both immune-related, were clinically relevant in FVPTC.

(iii) RAET-family members, also immune-related, were clinically relevant in TCPTC.

Collectively, our data suggest that dysregulation of both cancer and immune associated genes defines the gene expression landscapes of PTC variants, but different cancer or immune related genes may drive the phenotype of each variant.

The authors focused in Immune Associated genes (IA) and Cancer Associated genes (CA). As we shall note, there is a question as to what is meant by "cancer related genes". Namely, as we shall delineate, are these genes in the thyroid cells, are they dispositive, and how do they do what is supposed to be done? The authors further state:

In this study, we analyzed the co-dysregulation of immune-associated (IA) and cancer-associated (CA) genes in the different subtypes of PTC using a multi-scale approach, using data provided by The Cancer Genome Atlas (TCGA). We first: identified gene dysregulation on the scale of RNA expression. We then examined these dysregulations for significance on the scales of clinical variables correlations, copy number variation/aneuploidy correlations, mutation correlations, and micro-RNA regulation to identify key IA and CA genes that differentiate PTC subtypes from each other. In addition, on a pathways scale, we clustered IA and CA genes based on function to determine coordinated dysregulations of biological processes that define each PTC subtype.

The general conclusions reached by the authors are:

We conclude that several immune and cancer-associated genes and pathways define an RNA-expression dysregulation landscape that is unique to each individual PTC variant. Specifically,

(i) MUC1, S100-family genes, FN1, and several CA pathways characterize CTPC;

(ii) APLN, IL16, MAPK signaling, and several IA pathways characterize FVTPC; and

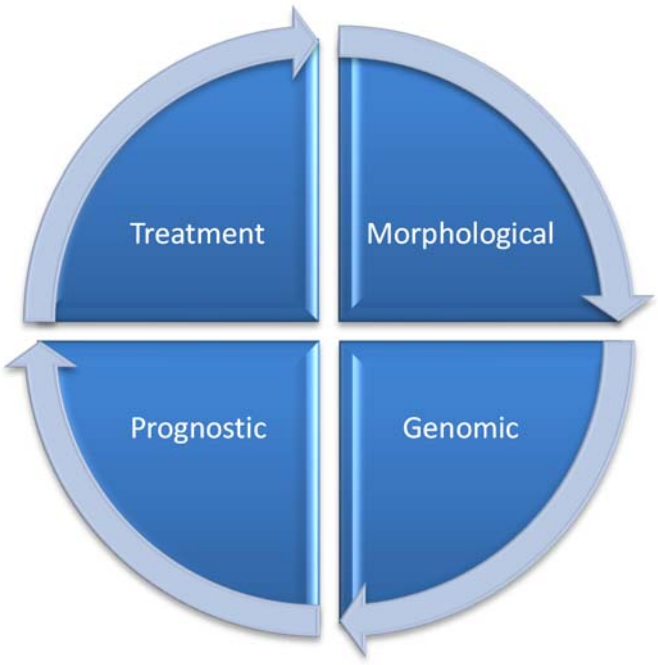
(iii) RAET-family genes and the antigen presentation pathway characterize TCPTC.

We also implicate miRNAs in the downregulation of certain genes and present a possible mechanism for miRNA-mediated suppression of TG.

The issue here is that there is an attempt to determine a genomic test to ascertain what the variant is of the PTC. Morphologically we have characterized them but genomically they still stand for added clarification.

1.2 A PRINCIPLE?

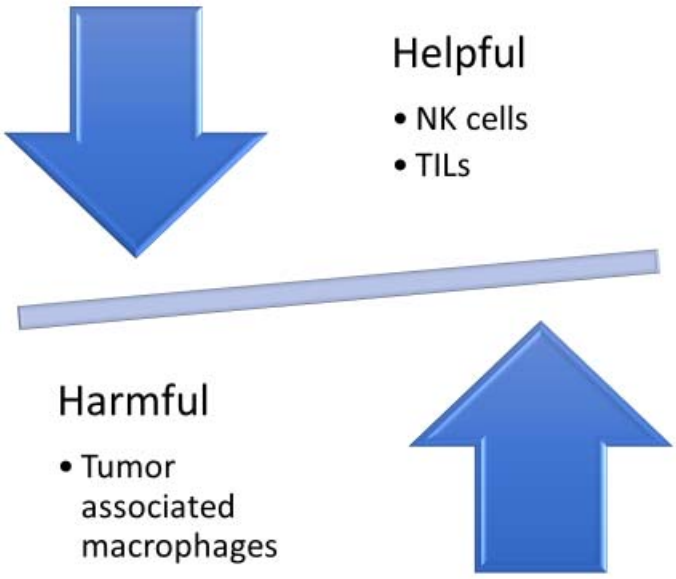
The principle we are attempting to examine is the nexus between the genetic differences and the morphological presentation. The ancillary issue that of the relationship between prognosis, and ultimately treatment, and the genomic presentation. Namely we depict below the issue at hand:



Currently the approach is to see the genomic as separate from the histological, and prognosis and treatment may be different based upon which of the two initial interpretations are relied upon. For example, if we have a micro FVPTC which is encapsulated but lacking any genetic deletions or fusions, but the histological is FVPTC and not NIFTP, then what should we call it and what should we do?

1.3 IMMUNE VERSUS CANCER

The authors indirectly present and interesting question. We know that genetic changes, breaks, fusions etc., result in malignancy. These are considered the cancer associated malignancies, a bit of an overuse of the term. Perhaps Cancer Associated would better be Genetic Associated, but I leave that to the authors. The second observation is that the immune system has a way of taking small genetic changes and either eliminating the bad cells or in some cases protecting or enhancing them. Consider the effects of tumor associated macrophages, TAMs. The immune associated can be helpful or harmful.



Thus, the immune system may help or it may hinder. Its hindering may be the result of further enhancement or activation of the tumor cells or the protection of those cells in some form of stroma. Thus the simplicity of seeing the immune system as all helpful should be looked at more carefully.

2 PTC VARIANTS

We briefly summarize key points on papillary thyroid cancers, PTC. From Nikiforov et al. (Table 13.6) we have a table shown below depicting the variations of papillary thyroid cancers. These are primarily histological descriptives.

Variant	***Diagnostic Criteria***	***Prevalence among Papillary Carcinomas (%)***
Papillary microcarcinoma	Size 1 cm or less	40
Infiltrative follicular variant	>50/% follicular growth pattern No well-formed papillae Infiltrative border	5-20
Encapsulated follicular variant[4]	>50% follicular growth pattern No well-formed papillae Encapsulation of clear demarcation Either invasion or 30-50% solid) trabecular or insular growth or 3 mitoses per 10 HPF or tumor necrosis	5-7
Tall cell variant	>30% tall columnar cells with height two to three times their width Abundant deeply eosinophilic cytoplasm	5-10
Solid variant	>50% solid, trabecular or insular growth	1-3
Diffuse sclerosing variant	Diffuse tumor growth within gland Abundant fibrosis Extensive lymphocytic infiltration Numerous psammoma bodies[5] Squamous metaplasia	1-2
Columnar cell variant	Columnar cells with nuclear stratification	0.2-1
Oncocytic (Hurthle cell) variant	>50% cells with oncocytic cytoplasm	<1
Warthin-like variant	Dense lymphocytic infiltration in papillary stalks Cells with oncocytic cytoplasm	<1
Clear cell variant	>50 % cells with clear cytoplasm	<1
Cribriform-morular variant	Cribriform growth pattern Morules	<1
Hobnail variant	>50% cells with hobnail features	<1

[4] *Follicular Variant This variant of papillary carcinoma is characterized by an exclusively or predominantly follicular growth pattern and lack of any well-formed papillae. The only other architectural pattern allowed is solid/trabecular/insular pattern present as a minor component. Important subclassification of the follicular variant of papillary carcinomas is into the infiltrative follicular variant and encapsulated/well-circumscribed follicular variant… Finally, in 2016, the latter group of encapsulated/well-delineated follicular variant papillary carcinomas was reevaluated and split into a borderline, very low-risk tumors named "noninvasive follicular thyroid neoplasm with papillary-like nuclear features" (NIFTP) and typically invasive encapsulated follicular variant papillary carcinomas*

[5] A psammoma body is a round collection of calcium

Variant	***Diagnostic Criteria***	***Prevalence among Papillary Carcinomas (%)***
Papillary carcinoma with desmoid-type fibromatosis/fasciitis-like stroma	Abundant cellular stroma resembling fibromatosis or nodular fasciitis	<1

Nikiforov et al in Fig 13.27 further summarizes the variants of PTC. With the exception of BRAF V600 and RAS they are also descriptive.

		Classic PTC	***Infiltrative FV PIC***	***Encapsulated FV PTC***	***NIFTP***	***Follicular carcinoma***
Histologic features	Papillae	+	-	-	-	-
	Nuclear features of PTC	+	+	+	+	-
	Encapsulation	-/+	-	+	+	+
	Invasion	+/-	+	+	-	+
Tumor spread		Distant	Distant	Distant[6]	None	Distant
Main mutation		*BRAF* V600E	*BRAF* V600E	*RAS*	*RAS*	*RAS*

Nikiforov et al have noted for PTC in general the following characteristics (see Table 13.5 as modified):

1. Infiltrative border

2. Architecture Papillary growth pattern

3. Nuclear features including[7]

> *I. Size and shape • Nuclear enlargement, crowding and overlapping • Nuclear elongation*
> *II. Membrane irregularities • Irregular nuclear contours • Nuclear grooves • Nuclear pseudoinclusions*
> *III. Chromatin characteristics • Chromatin clearing*

4. Psammoma bodies

5. Tumor fibrosis

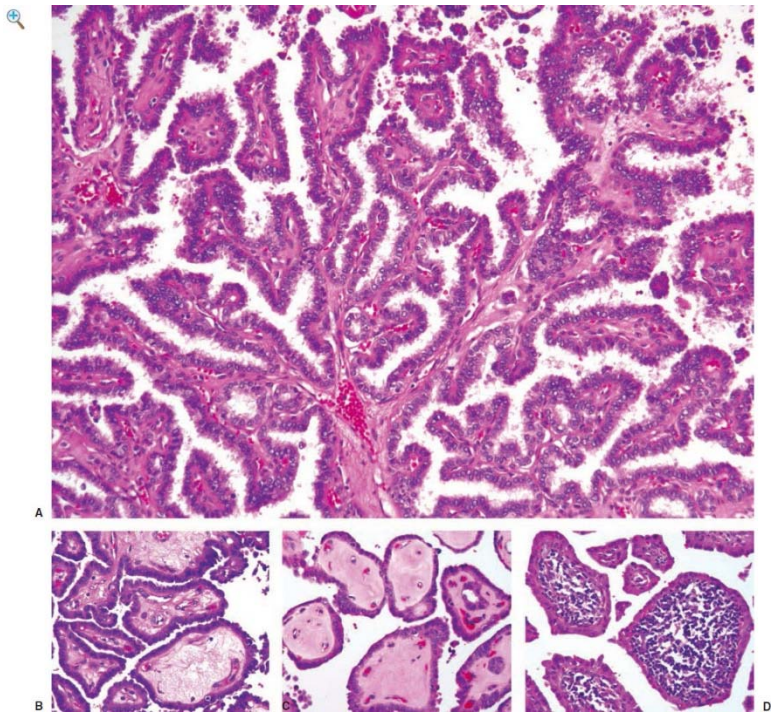
We now consider the three classes discussed in the introduction.

[6] Nikiforov et al note that if no invasion is noted, NIFTP may be excluded and the diagnosis of FVPTC may be given based upon: *In rare situations when invasion is absent, either 30% to 50% of solid/trabecular/insular growth pattern or high-grade features (tumor necrosis or high mitotic activity) should be found.*

[7] Note that these are the major nuclear features that one must look for.

2.1 CPTC

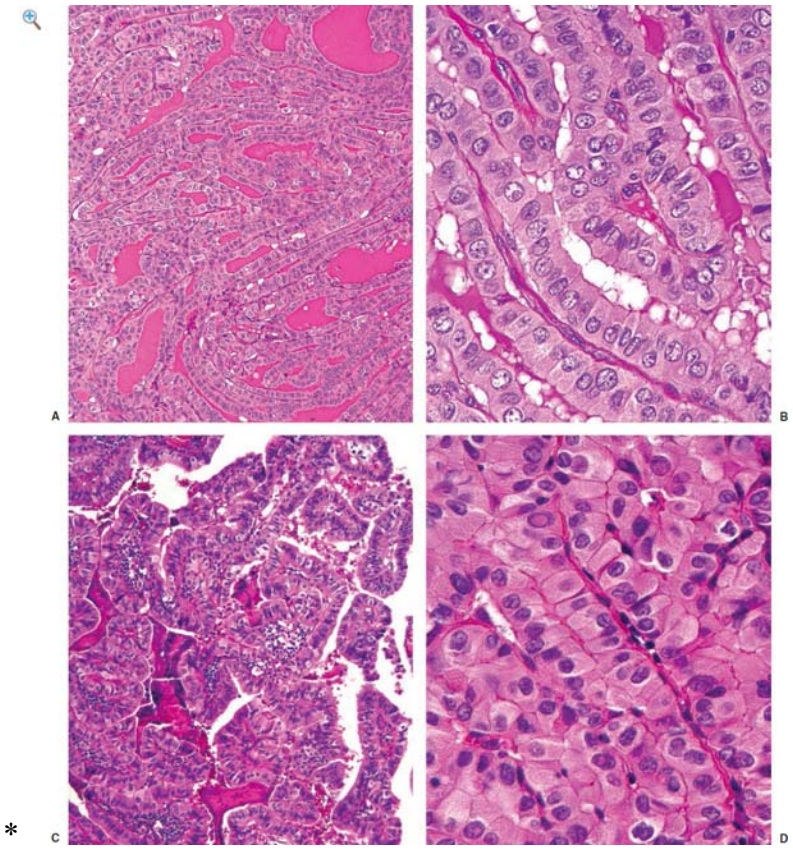
Papillary carcinoma is a well-differentiated malignant tumor of thyroid follicular cells that show a set of characteristic nuclear features. Either papillary growth or invasion is usually required for the diagnosis. An example of what could best be called classic papillary growth is shown below from Nikiforov et al:



Specifically, CPTC has well noted papilla as well as the clearly observed nuclear characteristics.

2.2 TALL CELL VARIANT

The Tall Cell Variant, TCPTC, is simply a PTC with longer than wider cells but it tends to be more highly aggressive. We show some examples from Nikiforov et al below.



As Nikiforov et al note:

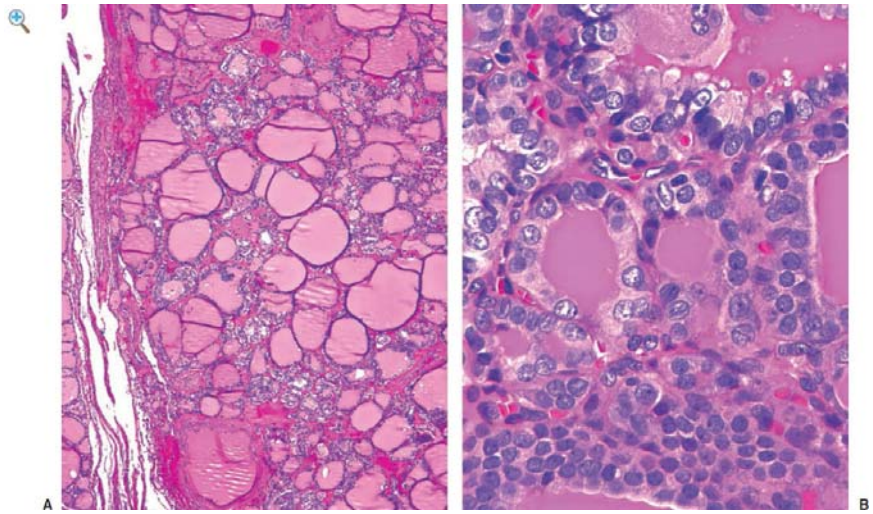
The tall cell variant is characterized by tall tumor cells, whose height is two to three times their width, and presence of abundant, deeply eosinophilic cytoplasm. The 2017 WHO Classification requires 30% or more of tumor cells to have such appearance, and such an approach is adopted by many others, although some pathologists require >50% of cells to have the characteristic microscopic features. The differences also exist in the requirements of cell height:width ratio, which is accepted as 2:1 by some and 3:1 by others.

Tall cell variant comprises 5% to 10% of all papillary carcinomas. It is exceedingly rare among pediatric tumors and seen with lower frequency in young patients. BRAF V600E mutation is very common and found in about 80% of these tumors. Some studies reported a higher frequency of TERT mutations in these tumors than in other papillary carcinomas. Some reports suggest that TP53 immunoreactivity is often seen in these tumors in contrast to the classic papillary carcinoma.

We have discussed the TCPTC previously as noted and it tends to be much more aggressive and infiltrative. The question we have also posed previously is; what are the genetic factors resulting in tall cell.

2.3 FVPTC

From Nikiforov et al (Fig 13.34) we have the FVPTC. The distinguishing characteristics are the follicular overall shape, lacking a preponderance of papilla, and the papillary nuclear features. This is an encapsulated form[8].



For the most part FVPTC is a more indolent variant with one subclass now considered an adenoma rather than a carcinoma. Small FVPTCs have been found in up to 40% of those over 65 years of age and it has been argued that it may very well be much higher. If a micro FVPTC (< 5 mm) is determined and there is no nodal involvement per a detailed ultrasound and after a reasonable period, say 9-12 months the thyroglobulin is within a normal range then this may very well be considered non-malignant. However, this may very well still be open to objection but it can be further argued that the objection may be reflective of the level of care more than the progression of the growth.

[8] Nikiforov et al note: *These tumors carry molecular alterations commonly characteristic of other follicular-patterned thyroid tumors, which are RAS mutations and PAX8/PPARG fusions. The same molecular profile is seen in NIFTP, with which the encapsulated follicular variant shares all histopathologic features with the exception of invasion, significant solid/trabecular/insular growth, or high-grade features. On the basis of these similarities, it is believed that these tumors are related, with NIFTP serving as a precursor lesion for at least a subset of encapsulated follicular variant papillary carcinomas.* We have seen encapsulated FVPTC with no discernable genetic mutations or fusions. Thus the genetic and morphological consistency is limited at best.

3 KEY GENES

We now examine the key genes that have been examined. The table below characterizes the three types the authors examine, the genes they argue have a unique PTC specific characteristic and whether they are cancer or immune related.

Cancer Type	Genes	Cancer Related	Immune Related
CPTC	MUC1 FN1 S100	X	
FVPTC	APLN IL16 CA/IA		X
TCPTC	RAET TNF RXR/RAR FCGR	X	X

We now present detains on each gene and whether the expression as related to the above are up or down. This will provide a baseline for which we can then examine each in more detail.

Gene	***Description***	***Up/Down Regulated***
MUC1[9]	This gene encodes a membrane-bound protein that is a member of the mucin family. **Mucins are O-glycosylated proteins that play an essential role in forming protective mucous barriers on epithelial surfaces.** These proteins also play a role in intracellular signaling. This protein is expressed on the apical surface of epithelial cells that line the mucosal surfaces of many different tissues including lung, breast stomach and pancreas. This protein is proteolytically cleaved into alpha and beta subunits that form a heterodimeric complex. **The N-terminal alpha subunit functions in cell-adhesion and the C-terminal beta subunit is involved in cell signaling.** Overexpression, **aberrant intracellular localization, and changes in glycosylation of this protein have been associated with carcinomas**. This gene is known to contain a highly polymorphic variable number tandem repeats (VNTR) domain. Alternate splicing results in multiple transcript variants	Up

[9] https://www.ncbi.nlm.nih.gov/gene/4582

Gene	*Description*	*Up/Down Regulated*
FN1[10]	This gene encodes **fibronectin, a glycoprotein present in a soluble dimeric form in plasma, and in a dimeric or multimeric form at the cell surface and in extracellular matrix.** The encoded preproprotein is proteolytically processed to generate the mature protein. Fibronectin is involved in **cell adhesion and migration processes including embryogenesis, wound healing, blood coagulation, host defense, and metastasis.** The gene has three regions subject to alternative splicing, with the potential to produce 20 different transcript variants, at least one of which encodes an isoform that undergoes proteolytic processing. The full-length nature of some variants has not been determined.	Up
S100A4[11]	The protein encoded by this gene is a member of the S100 family of proteins containing 2 EF-hand calcium-binding motifs. S100 proteins are **localized in the cytoplasm and/or nucleus of a wide range of cells,** and involved in the regulation of a number of **cellular processes such as cell cycle progression and differentiation**. **S100 genes include at least 13 members which are located as a cluster on chromosome 1q21.** This protein may function in motility, invasion, and tubulin polymerization. Chromosomal rearrangements and altered expression of this gene have been **implicated in tumor metastasis**. Multiple alternatively spliced variants, encoding the same protein, have been identified.	UP
APLN[12]	This gene encodes a peptide that functions as an **endogenous ligand for the G-protein coupled apelin receptor.** The encoded preproprotein is proteolytically processed into biologically active C-terminal peptide fragments. These peptide fragments activate different tissue specific signaling pathways that regulate diverse biological functions including fluid homeostasis, cardiovascular function and insulin secretion. This protein also functions as a coreceptor for the human immunodeficiency virus.	Up
IL 16[13]	The protein encoded by this gene is a **pleiotropic cytokine that functions as a chemoattractant, a modulator of T cell activation, and an inhibitor of HIV replication.** The signaling process of this cytokine is mediated by CD4. The product of this gene undergoes proteolytic processing, which is found to yield two functional proteins. The cytokine function is exclusively attributed to the secreted C-terminal peptide, while the N-terminal product may **play a role in cell cycle control.** Caspase 3 is reported to be involved in the proteolytic processing of this protein. Alternate splicing results in multiple transcript variants.	Down

[10] https://www.ncbi.nlm.nih.gov/gene/2335

[11] https://www.ncbi.nlm.nih.gov/gene/6275

[12] https://www.ncbi.nlm.nih.gov/gene/8862

[13] https://www.ncbi.nlm.nih.gov/gene/3603

Gene	*Description*	*Up/Down Regulated*
RAET1E[14]	This gene belongs to the RAET1 family, which consists of major histocompatibility complex (MHC) class I-related genes located in a cluster on chromosome 6q24.2-q25.3. This and RAET1G protein differ from other RAET1 proteins in that they have type I membrane-spanning sequences at their C termini rather than glycosylphosphatidylinositol anchor sequences. **This protein functions as a ligand for NKG2D receptor, which is expressed on the surface of several types of immune cells, and is involved in innate and adaptive immune responses.** Alternatively spliced transcript variants encoding different isoforms have been found for this gene.	Up
RAET1G[15]	This gene encodes a member of the major histocompatibility complex (MHC) class I family of proteins. Although the encoded protein includes C-terminal transmembrane and cytoplasmic domains, proteolytic processing results in the removal of these domains and subsequent tethering to the plasma membrane by a glycosylphosphatidylinositol (GPI)-anchor. **The encoded protein is one of several related ligands of the natural killer group 2, member D (NKG2D) receptor, which functions as an activating receptor in innate and adaptive immunity.** This gene is present in a gene cluster on chromosome 6.	Up
NKG2D[16]	Natural killer (NK) cells are lymphocytes that can mediate lysis of certain tumor cells and virus-infected cells without previous activation. They can also regulate specific humoral and cell-mediated immunity. NK cells preferentially express several calcium-dependent (C-type) lectins, which have been implicated in the regulation of NK cell function. **The NKG2 gene family is located within the NK complex, a region that contains several C-type lectin genes preferentially expressed in NK cells.** This gene encodes a member of the NKG2 family. The encoded transmembrane protein is characterized by a type II membrane orientation (has an extracellular C terminus) and the presence of a C-type lectin domain. It binds to a diverse family of ligands that include MHC class I chain-related A and B proteins **and IL-16 binding proteins, where ligand-receptor interactions can result in the activation of NK and T cells.** The surface expression of these ligands is important for the recognition of stressed cells by the immune system, and thus this protein and its ligands are therapeutic targets for the treatment of immune diseases and cancers. Read-through transcription exists between this gene and the upstream KLRC4 (killer cell lectin-like receptor subfamily C, member 4) family member in the same cluster.	

[14] https://www.ncbi.nlm.nih.gov/gene/135250

[15] https://www.ncbi.nlm.nih.gov/gene/353091

[16] https://www.ncbi.nlm.nih.gov/gene/22914

Gene	*Description*	*Up/Down Regulated*
TNF[17]	This gene encodes a multifunctional proinflammatory cytokine that belongs to the tumor necrosis factor (TNF) superfamily. This cytokine is mainly secreted by macrophages. It can bind to, and thus functions through its receptors TNFRSF1A/TNFR1 and TNFRSF1B/TNFBR. This cytokine is involved in the regulation of a wide spectrum of biological processes including cell proliferation, differentiation, apoptosis, lipid metabolism, and coagulation. This cytokine has been implicated in a variety of diseases, including autoimmune diseases, insulin resistance, and cancer. Knockout studies in mice also suggested the neuroprotective function of this cytokine.	
FCGR2A[18] Note there are multiple FCGR variants FCGR3A[19] FCGR2B[20] FCGR3B[21] FCGR2C[22]	This gene encodes one member of a **family of immunoglobulin Fc receptor genes found on the surface of many immune response cells.** The protein encoded by this gene is a cell surface receptor found on phagocytic cells such as macrophages and neutrophils, and is involved in the process of phagocytosis and clearing of immune complexes. Alternative splicing results in multiple transcript variants.	Down

[17] https://www.ncbi.nlm.nih.gov/gene/7124

[18] https://www.ncbi.nlm.nih.gov/gene/2212

[19] https://www.ncbi.nlm.nih.gov/gene/2214

[20] https://www.ncbi.nlm.nih.gov/gene/2213

[21] https://www.ncbi.nlm.nih.gov/gene/2215

[22] https://www.ncbi.nlm.nih.gov/gene/9103

4 DETAILS

We now examine the details of each of the putative genes and consider the implications.

4.1 OVERALL GENETIC OBSERVATIONS

To again examine the statements of Chakladar et al regarding genes they state:

*(i) A total of 153 genes were significantly dysregulated **in all three subtypes** when compared to normal samples.*

(ii) 207 genes were dysregulated in both CPTC and TCPTC,

(iii) 18 genes were dysregulated in both FVPTC and CPTC, and

(iv) 20 genes are dysregulated in both FVPTC and TCPTC.

This suggests that the landscape of IA (immune) and CA (cancer) dysregulation is most similar between TCPTC and CPTC. Interestingly,

(v) 300 genes were dysregulated solely in TCPTC, while only

(vi) 22 were dysregulated solely in CPTC,

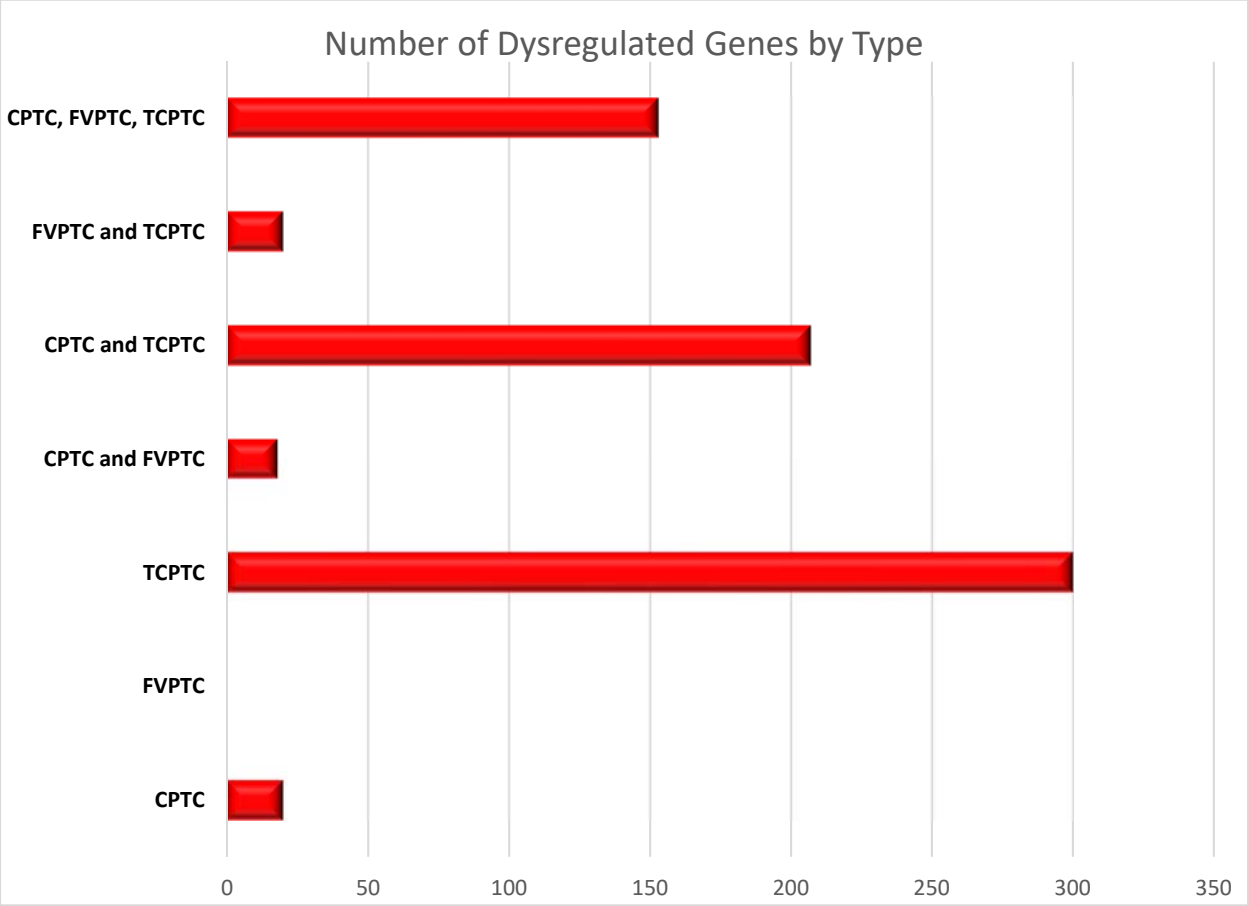
suggesting that while TCPTC is most similar to CPTC in gene dysregulation, it is still relatively distinct compared to the other subtypes.

A larger number of genes are dysregulated in TCPTC than in other subtypes. From volcano plots of differential expression results, we observe that the fold changes of most dysregulated genes in FVPTC were not significant, whereas the fold changes of IA and CA genes are much higher in the other two subtypes.

These results suggest that FVPTC and normal tissue are relatively similar, and the lack of high fold-change dysregulation may explain the relatively benign phenotype of FVPTC. Due to the important differences between the newly classified variants of FVPTC, we attempted to investigate immune-associated elements that may differ between them.

TCGA data was limited in this regard as FVPTC patients were not further classified into subtypes. We looked to sequencing data from a previous study,… and performed differential expression analysis on niFVPTC vs iFVPTC samples. The genotypes of both sub-variants were similar, as evidenced by the small number of differentially expressed genes. Only a limited number of IA genes were found to be significantly dysregulated between the two sub-variants. We therefore found that the determining factor of FVPTC morphology may not be related to immune-associated elements.

We depict the above in the following figure.



We now examine the details of each PTC variation.

4.2 CPTC

Classical PTC appears as a proliferation with papilla and the nuclear characteristics. It is a common presentation as we have shown.

4.2.1 MUC1

MUC1 has been studied in several cancers. From Siragusa et al we have the following general description of this gene and its functions:

In normal secretory epithelial cells, MUC1 is expressed as a transmembrane glycoprotein that provides protection against pathogens and shows cell signaling ability. Following synthesis as a single polypeptide and cleavage in the endoplasmic reticulum, MUC1 is expressed on cell membrane as a heterodimer. The MUC1 NH2-terminal subunit (MUC1-N) consists of variable numbers of 20–amino acid tandem repeats that are modified by O-glycans. … With transformation and loss of polarity, MUC1 is found at high levels in the cytosol and throughout the cell membrane of carcinoma cells. MUC1 oncoprotein overexpression is sufficient to

attenuate oxidative-induced and genotoxic stress–induced apoptosis in most cancers. In addition, recent findings have revealed that diverse carcinoma cells express the MUC1-C in mitochondria or in the nucleus in association with the Wnt effector h-catenin. MUC1 interacts with members of the ErbB family of receptor tyrosine kinases and with the fibroblast growth factor receptor 3.

Stimulation of such receptors induces c-Src–dependent tyrosine phosphorylation of the MUC1-CD on a YEKV motif and thereby results in nuclear localization of MUC1 and h-catenin or heat shock protein (Hsp) 90–mediated targeting of MUC1 to mitochondria.

Recently, it has been reported that MUC1-induced transformation of fibroblasts is due to activation of the antiapoptotic PI3K/ Akt and Bcl-xL pathways.

By contrast, in colon and breast carcinoma cells, ***MUC1 cytoplasmic domain activates the FOXO3a transcription factor that induces oxidant scavenging and DNA repair in a survival response to oxidative stress; this observation is due to the reduced activation of PI3K/Akt pathway and thereby to the decreased FOXO3a phosphorylation.***

These findings collectively suggest that the close cross-talk occurring between MUC1 and Akt signal transduction pathway depends on the cell context. ***MUC1 oncoprotein overexpression has been proposed as a key molecular event in the pathogenesis of aggressive PTC, thus designating it as a prognostic marker and potential therapeutic target for this disease***

In effect, MUC1 can be a powerful intermediate in key pathway operations. They continue:

MUC1 interacts directly with the Wnt pathway *effector hcatenin and glycogen synthase kinase 3h. GSK3h phosphorylates MUC1 cytoplasmic domain on serine in a SPY site, decreasing the interaction with h-catenin. Conversely, tyrosine phosphorylation of the SPY site increases the formation of complexes MUC1-h-catenin. MUC1-h-catenin complexes localize in the nucleus of several human carcinoma cells and function as coactivators of Tcf/LEF-1 target gene transcription.*

In thyroid cancer, h-catenin plays a direct role in the dedifferentiation commonly observed in late-stage disease. *Activating mutations in h-catenin have been shown in late-stage thyroid tumors and lead to h-catenin nuclear localization and poor prognosis.* ***The considerable activation of the PI3K/Akt pathway in thyroid cancer cells results in GSK3h phosphorylation and deactivation and subsequent h-catenin up-regulation****. Based on these remarks, MUC1-h-catenin complexes might form 4 and localize in the nucleus of thyroid cancer cells, contributing to the malignant phenotype. In addition, overexpression of MUC1 in the absence of GSK3h activity might inhibit the formation of the E-cadherin-h-catenin complexes and favor cell migration and metastasis formation by weakening adherent junctions.*

Kato et al note:

Mucus lining the airway lumen serves as a major protective barrier for the lung. Although many airborne particles and toxic chemicals are trapped in the mucus layer and continuously cleared by the mucociliary escalator, some have evolved mechanisms to circumvent this barrier. Any abnormalities in the quantity and/or quality of mucus can cause serious pulmonary

complications, often leading to the death of patients with diseases such as cystic fibrosis, bronchiectasis, chronic obstructive pulmonary disease, and asthma. Mucins are the major glycoproteins present in mucus, and their presence contributes to the viscoelastic properties of mucus. Twenty-one mucin genes have been identified in humans, 14 of which are expressed in the respiratory tract. The translated products of these mucin genes are broadly classified as either gel-forming, secreted, or membrane-tethered mucins. All mucin proteins are heavily glycosylated, primarily through the post-translational attachment of O-linked glycans, and all mucins contain variable numbers of tandemly repeated amino acid sequences with a high content of serine, threonine, and proline residues. Among the membrane-tethered mucins in the respiratory tract—primarily MUC1, MUC4, and MUC16—MUC1 has been the best characterized.

There are several mucins and MUC1 is but one. They tend to be cell protective devices which may inhibit immune cells attacking and eliminating aberrant cells.

Thus, MUC1 can result in the loss of differentiation and thus significant malignancy as well as the change in catenin. As Hu et al have noted:

***MUC1 is an important cancer-associated antigen gene and plays an important role in the incidence and development of malignant tumors**. Because of the high expression of MUC1, the distribution of polarity is destroyed, and the adhesion between tumor cells is reduced, resulting in the **adhesion of tumor and normal cells, immune escape, tumor cell proliferation, tumor invasion, and lymphatic metastasis**.*

Many studies on MUC1 have focused on its role in breast cancer; however, few studies that relate have been published. to thyroid cancer c-myc is a common proto-oncogene, which can promote cell proliferation and induce cell apoptosis, and plays a role in regulating cell growth, differentiation, and malignant transformation.

*In particular, offset or excessive c-myc expression plays an important role in thyroid cancer development. This study found that **the positive rates of MUC1 and c-myc protein expression in cancer tissues from patients with PTC were significantly higher than those in patients with NTT and NG**, which indicated that there were obvious differences in their expression in benign and malignant thyroid lesions. Therefore, it might be considered that MUC1 and C- myc can not only serve as a reference indicator of thyroid cancer in PTC, but might also play a role in the differential diagnosis of thyroid cancer and benign thyroid lesions.*

*Furthermore, MUC1 expression in patients with local invasion and lymph node metastasis was also shown to be higher than that in patients without invasion and lymphatic metastasis, suggesting that MUC1 played a role in promoting the invasion and lymphatic metastasis of thyroid cancer; …. We also found that c-myc expression was related only to lymph node metastasis; therefore, it might be considered as a basis to determine the likelihood of poor prognosis. **Together, these results suggest that the detection of MUC1 and c-myc expression in patients would be helpful in the determination of the biological behavior of thyroid cancer and the correct prediction of prognosis, which can be used to guide clinical treatment.***

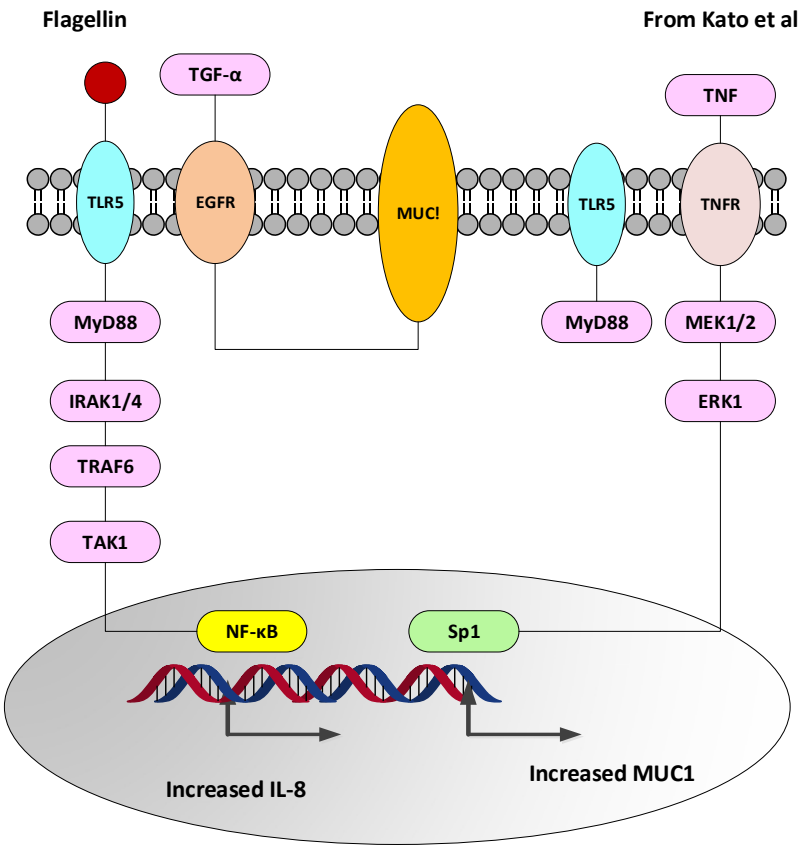
The latter observation may be a lead to a therapeutic path. At present there does not seem to be a great deal of work on that topic. However, Hollingsworth and Swanson have noted:

Mucins — large extracellular proteins that are heavily glycosylated with complex oligosaccharides — establish a selective molecular barrier at the epithelial surface and engage in morphogenetic signal transduction. Alterations in mucin expression or glycosylation accompany the development of cancer and influence cellular growth, differentiation, transformation, adhesion, invasion and immune surveillance. Mucins are used as diagnostic markers in cancer, and are under investigation as therapeutic targets for cancer.

Cancer cells, especially adenocarcinomas, express aberrant forms or amounts of mucins. The expression of distinct oligosaccharide structures, together with differential glycosylation of mucin core proteins, confers on tumour cells an enormous range of potential ligands for interaction with other receptors at the cell surface. Cancer cells might use mucins in much the same way as normal epithelia — for protection from adverse growth conditions and to control the local molecular microenvironment during invasion and metastasis. Mucins are hypothesized to contribute to tumour invasion by simultaneously disrupting existing interactions between opposing cells (anti-adhesion) and establishing new ligands for interaction between the invading cell and the adjoining cells (adhesion). Mucins could contribute to the regulation of differentiation and proliferation of tumour cells, through ligand–receptor interactions (for example, between MUC4 and ERBB2 (also known as HER2/neu) and morphogenetic signal transduction.

They further examine the MUC1 pathways and drivers. MUC1 also has the ability to eliminate adhesion of cells and perhaps facilitate EMT[23]. We present the pathways involved below based upon Kato et al:

[23] https://www.researchgate.net/publication/330222973_EMT_and_Cancers



What may be interesting regarding MUC1 is the possibility that it is the excess product covering the cell which may give rise to the papilla like structure, namely the dislocation of well-formed follicular cells. This hypothesis may be corroborated by some of the recent work by Patel et al who note:

MUC1 is a transmembrane epithelial cell surface glycoprotein. It belongs to the family of mucin proteins which are expressed by various epithelial cell types. Mucins are multifaceted glycoproteins that provide lubrication of epithelial cell surfaces, prevent tissue dehydration, protect cells from proteolytic degradation and constitute a barrier against infection. They have a central role in maintaining homeostasis and promoting cell survival. Cell surface associated mucins, such as MUC1, are bound to cells by an integral transmembrane domain and have relatively short cytoplasmic tails that associate with cytoskeletal elements, cytosolic adaptor proteins and/or participate in signal transduction.

They may serve as cell surface receptors and sensors and conduct signals in response to external stimuli that lead to coordinated cellular responses that include proliferation, differentiation, apoptosis or secretion of specialized cellular products. ***Cancer cells might use mucins in much the same way as normal epithelia, for protection from adverse growth conditions and to control the local microenvironment during invasion and metastasis.*** *MUC1 is over-expressed and aberrantly glycosylated in almost all human adenocarcinomas, including >90% of breast, ovarian, pancreatic, colorectal, lung, prostate and gastric carcinomas and has been implicated in their pathogenesis.*

MUC1 expression has also been demonstrated in nonepithelial cancer cell lines, such as astrocytoma, melanoma and neuroblastoma, as well as hematological malignancies such as multiple myeloma and some Bcell non-Hodgkin lymphomas. Interestingly this comprises >50% of all cancers in humans. MUC1 is ubiquitously expressed over the entire cell membrane in cancer cells, while it is restricted to only the apical surface in normal epithelial cells.

Thus, MUC1 appears to be a powerful intermediary in the protection and progression of a multiplicity of cancers.

4.2.2 FN1

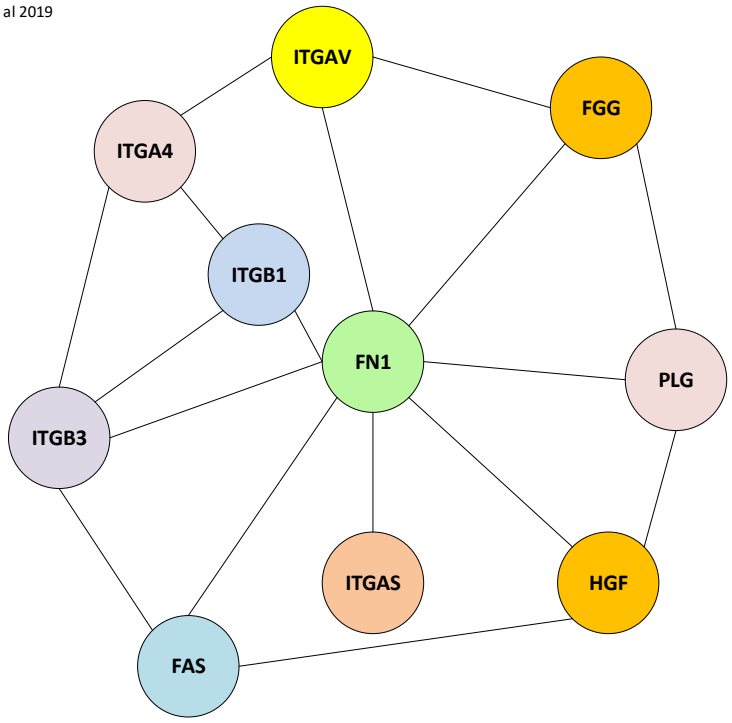
FN1 is a gene related to various fibronectin functions. As Li et al have noted:

Fibronectin 1 (FN1) is a member of the glycoprotein family that is widely expressed by multiple cell types. ***FN1 plays a major role in cell adhesion, growth, migration and differentiation, and it is important for processes such as wound healing and embryonic development****.*

Degradation or organization of FN1 expression has been associated with cancer progression, such as squamous cell carcinoma, nasopharyngeal carcinoma, ovarian cancer, renal cancer and thyroid cancer*. Recent studies have shown that increased expression of FN1 in tumor cells is negatively correlated to the prognosis of patients. Furthermore, researcher suggested that increased* ***FN1 expression may be associated with lung tumor growth/survival*** *and resistance to therapy. Our studies showed that FN1 survived from melanoma metastasis and its expression was upregulated in metastatic tumor cells as compared to primary tumor cells.*

We depict the related genes of FN1 interaction as discussed in the work of Li et al.

See Li et al 2019



Again for the papillary structure, along with MUC1, FN1 may significantly alter the normal follicular structure allowing for papilla during proliferation.

4.2.3 S100

The S100 family is a large family of genes. They have been studied in a variety of fields and their influence on cancers can be significant. S100 genes have a wide range of functions many of which may result in aggressive malignancies if loss of control is effected.

As Chen et al note:

S100 protein family has been implicated in multiple stages of tumorigenesis and progression. ***Among the S100 genes, 22 are clustered at chromosome locus 1q21, a region frequently rearranged in cancers****. S100 protein possesses a wide range of intracellular and extracellular functions* ***such as regulation of calcium homeostasis, cell proliferation, apoptosis, cell invasion and motility, cytoskeleton interactions, protein phosphorylation, regulation of transcriptional factors, autoimmunity, chemotaxis, inflammation and pluripotency****.*

Many lines of evidence suggest that altered expression of S100 proteins was associated with tumor progression and prognosis. ***Therefore, S100 proteins might also represent potential tumor biomarkers and therapeutic targets.*** *In this review, we summarize the evidence connecting S100 protein family and cancer and discuss the mechanisms by which S100 exerts its diverse functions….The association between S100 proteins and cancer can also be explained by several observations:*

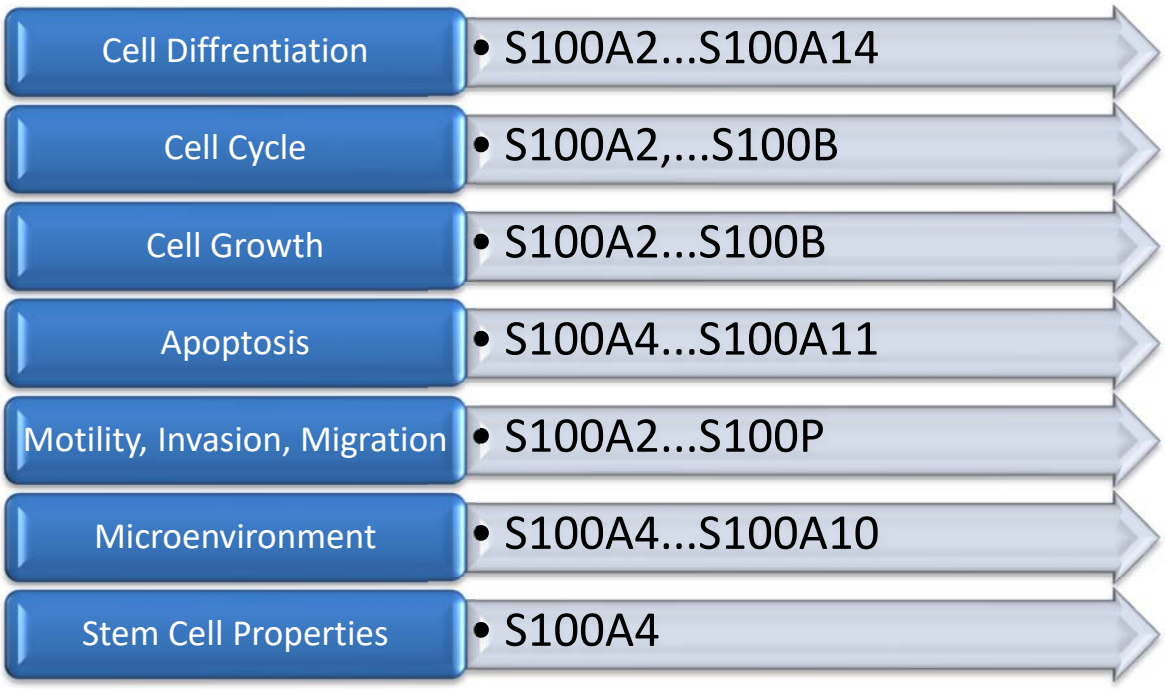
firstly, most of S100 genes are clustered on human chromosome 1q21, a region prone to genomic rearrangements, supporting that S100 proteins may be implicated in tumor progression.

Secondly, several S100 members show altered expression in various malignancies.

Finally, a number of S100 proteins have been shown to interact with and to regulate various proteins involved in cancer and exert different effects on specific target proteins such as NF-kB, p53, and p-catenin.

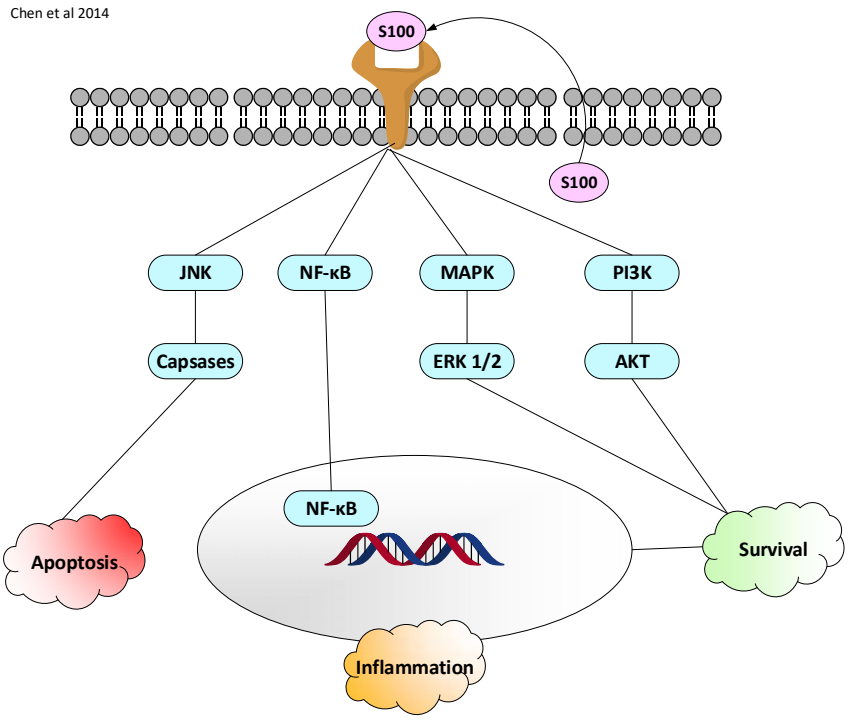
In this review we discuss the important ***roles of S100 proteins in tumorigenesis, cancer metastasis, tumor microenvironment, maintenance of pluripotency and their potential implications as biomarkers and prognostic factors.*** *We also discuss the underlying mechanisms by which S100 proteins involved in tumorigenesis and cancer progression. Elucidating the mechanisms of S100 signaling in cancer will increase our understanding of tumorigenesis and may lead to the identification of new therapeutic targets.*

S100 genes may thus be good targets for therapeutics as well as markers. The authors present some of the functions of the S100 family and the related specific members as we show below:



The authors further depict the pathway actions as shown below[24]:

[24] The authors note: *S100 proteins in RAGE signaling. S100 proteins can be secreted into the extracellular space, and crosslink with cell-surface receptor-RAGE and deliver signals inside the cell, thereby modulate cell survival, proliferation or apoptosis. Some S100 proteins (S100P, S100A8/A9, S100A12, S100A14, S100B) can interact with RAGE, subsequently activating the MAPK, PI-3K-AKT, and NF-kB signaling pathways, and thereby leading to the*



Of the S100 variants Chen et al note that for thyroid cancers S100A4 and S100A16 are the only specific ones attributed.

4.3 FVPTC

FVPTC is an interesting class of TC. They are identified by being follicular in assembly but papillary in nuclear and cellular structure. We have examined some of these issues of genes and morphology previously. Generally, the diagnosis is based upon the histological study. There is a growing interest in genetic profiling but the current set of profiles are limited.

4.3.1 MAPK Signalling

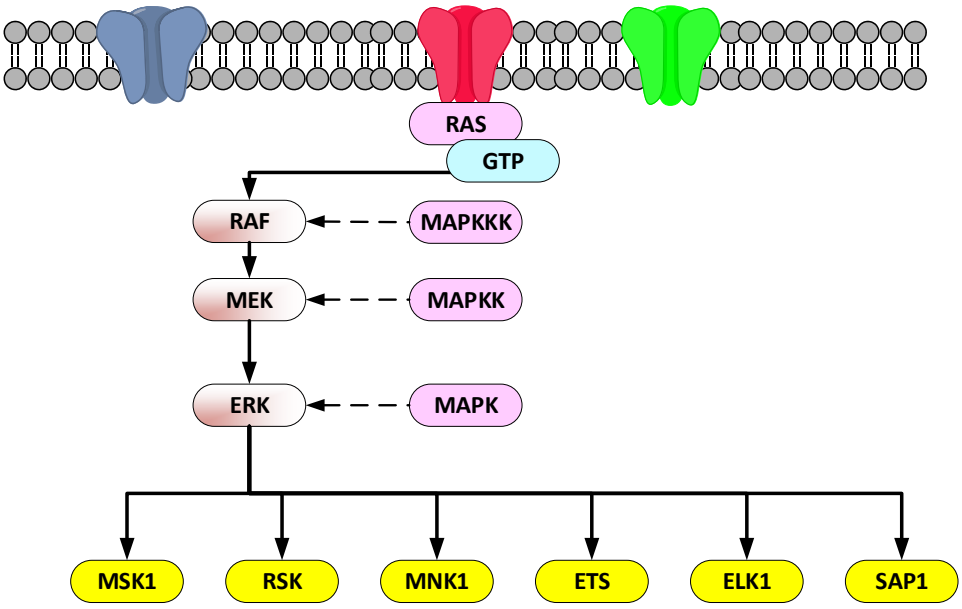
MAPK pathways are well known players in a multiplicity of cancers. They are well studied and also have a variety of therapeutics available for control. Chakladar et al. have noted regarding the MAPK pathways the following:

The most prominent mechanism studied is the point mutation of the BRAF proto-oncogene, which results in the expression of the BRAF-V600E mutant protein. This protein promotes tumorigenesis by activating the mitogen-activated protein kinase (MAPK) signaling pathway, a pathway crucial for cell proliferation and survival. This mutation has been shown to be present predominantly in CPTC and TCPTC patients and is correlated with an aggressive phenotype contrary to the slow-growing nature of PTC in general…

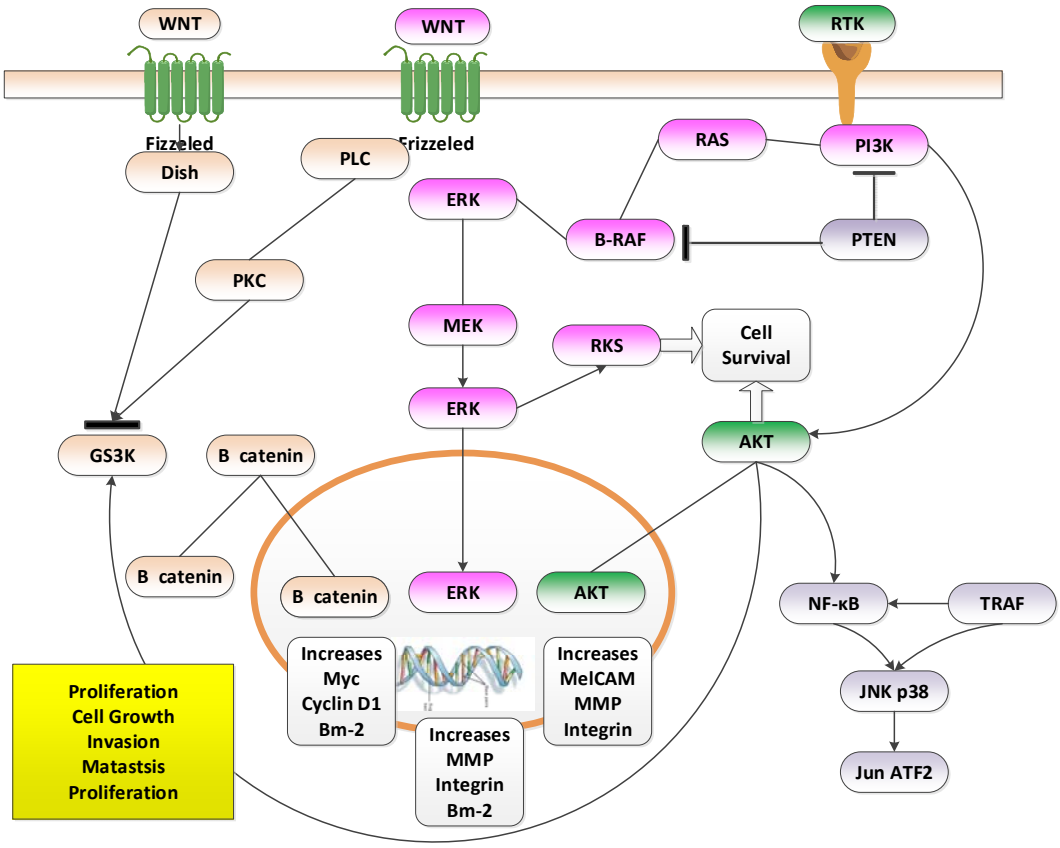
up-regulation of genes involved in cell survival and proliferation. In other cases, the apoptosis cascade is activated through the activation of JNK and caspases.

The second most prevalent mutation is on RAS and its isoforms, HRAS, NRAS, and KRAS. These mutations are dual activators of the MAPK and the phosphatidylinositol 3-kinase/Akt (PI3K/AKT) pathways, but more commonly activate the latter pathway in promoting tumorigenesis in FVPTC patients

Namely for FVPTC we see RAS mutations more than a BRAF mutation. To better grasp this issue we show the MAPK signally paths are shown below.



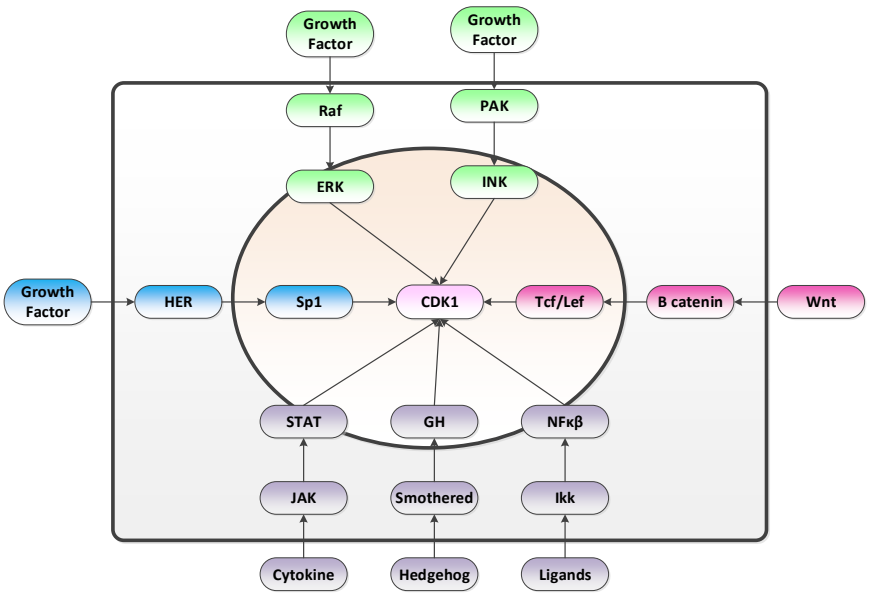
Key amongst the above for consideration in FVPTC is BRAF and the variant V600. The authors note for FVPTC as follows the RAS mutations. We show all such pathways below with their resulting effects.



The authors note:

We discovered that in contrast to genes in CPTC, dysregulated genes in FVPTC ***do not cluster functionally in relation to p53 effectors or the AP-1 transcription factor network. Instead, the CA pathways of MAPK signaling and TNF signaling as well as the IA pathways of T-cell/B-cell signaling and Jak-STAT signaling are most prominently dysregulated in FVPTC****.*

We show the JAK path below:



Note the JAK/STAT driver of CDK1, a proliferation driver in the cell cycle. However there appears to be a lack of clarity and specificity here. There are a significant number of pathways and drivers and thus a lack of on therapeutic targets. The above description may then tend to lay out some directions for therapeutics.

4.3.2 APLN

A second target is the APLN gene and its product. As Zhang et al have noted regarding APLN:

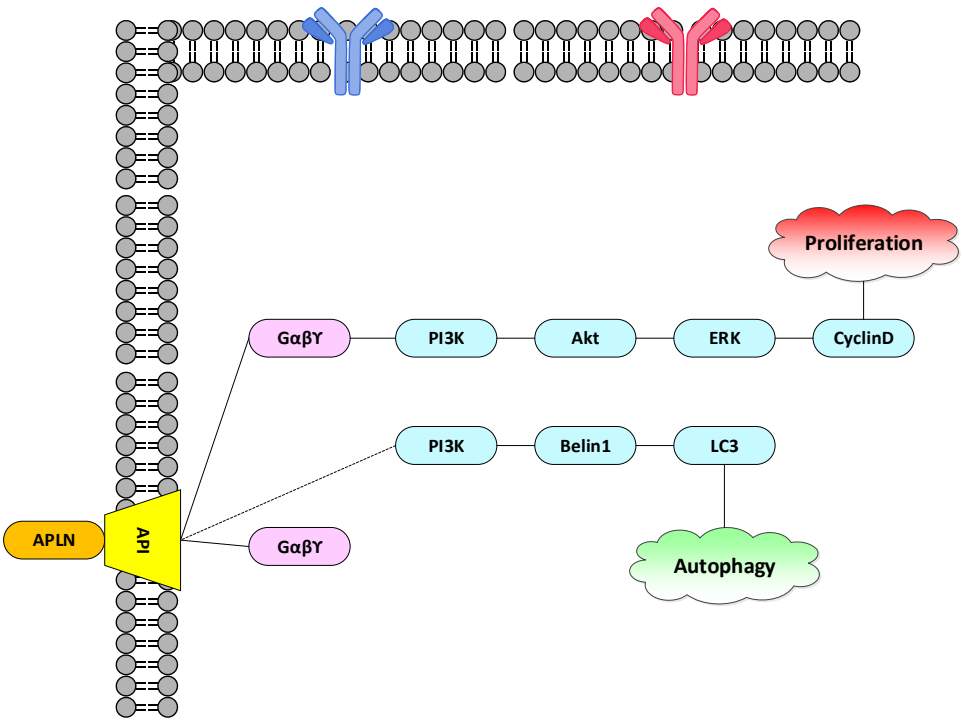
Apelin, the endogenous ligand for the APJ receptor (an orphan G-protein-coupled receptor), is a bioactive peptide that is expressed in a wide variety of tissues. The apelin gene (APLN) in humans is located on chromosome Xq25–26.1, which encodes a 77-amino acid prepropeptide that is cleaved into isoforms of varying lengths.

During the recent years, there is a mounting evidence that apelin has pleiotropic effects on lipid and glucose metabolisms and therefore is correlated with metabolic disorders including diabetes. On the other side, since the apelin/APJ system has high sequence homology to the angiotensin II/angiotensin receptor (AII/AT) system, it has gained much research interest regarding its role in blood pressure control.

By binding to APJ, apelin exerts a potent hypotensive effect via a nitric oxide-dependent mechanism in vivo. In accord with this, circulating apelin was reported to be decreased in patients with essential hypertension, suggesting an important role for the apelin/APJ system in human blood pressure regulation.

From KEGG we have a simplified version of APLN actions[25]:

[25] https://www.kegg.jp/pathway/hsa04371



Yu et al have detailed several functions of APLN:

1. The APLNR is highly expressed during mesendodermal differentiation
2. APLN binds mesodermal and endodermal progenitors in differentiating hESCs
3. Hemangioblast colony forming cells express APLNR.
4. APLN influences EB size and morphology
5. APLN increases hematopoietic gene expression
6. APLN augments hematopoiesis
7. APLN enhances the endothelial growth and hematopoietic colony forming potential of d6 CD34brKDRbr cells

APLN may thus be a driver for both angiogenesis and for cell morphology. The element of morphology is an interesting factor given the unique cellular nucleus presentation in FVPTC.

4.3.3 IL16

An immune factor associated with FVPTC is the cytokine IL-16. Interleukins are cytokine and as such signal other immune elements to go on the attack. The sources of IL-16 are lymphocytes, epithelial cells, eosinophils, CD8+ T cells and they attach to CD4 on Th cells.

As Curiel-Lewandrowski et al note:

The protein, pro–IL-16, is derived from the precursor protein (IL-16), comprised of 631 amino acids, and is present at very high levels in approximately 90%–97% of all T cells. After cell activation via the T cell receptor, precursor IL-16 is cleaved by caspase-3, which produces mature IL-16 (derived from the C-terminal 121 AA) and pro–IL-16. ***Mature IL-16 is well characterized as a CD4 ligand that induces chemotaxis and CD25 expression in CD4+ T cells. While IL-16 can function as a competence growth factor for normal primary T cells, it has been shown to function as a complete growth factor for T cell lines.***

IL-16 may thus be a significant anti-tumor factor for the activation and control of T cells. This may be significant in the control of aberrant cell growth as may be seen in FVPTC. However, the question is; what is the source of the IL-16? Is it the thyroid follicular cells and if IL-16 is suppressed is it via the follicular cell and by what pathway? In examining the proposition in the paper in question we question the source and in addition the mechanism for the suppression.

As Zheng et al have noted:

IL-16 plays different roles in different pathological processes as an immunomodulatory cytokine. ***As a chemokine, IL-16 drives the chemotactic movement of CD4+ Th cells, monocytes, and eosinophils toward sites of inflammation****,* ***promotes expression of the alpha chain of the IL-2 receptor, and activates CD4+ T cells synergistically with either IL-2 or IL-15.*** *Further, as a proinflammatory cytokine, IL-16* ***promotes inflammatory reactions by stimulating cytokine production by monocytes and mature macrophages****. In this context, IL-16 promotes the secretion of proinflammatory cytokines in allergic diseases.*

In AD patients, activated epidermal Langerhans cells, induced by allergens, secrete IL-16. IL-16 in turn recruits and activates ***dendritic cells, T cells, and eosinophils****. Therefore, it is evident that IgE-mediated inflammatory response and cell inflammation are related. In addition, allergen-induced eosinophils also secrete IL-16. This IL-16 in turn recruits more eosinophils, capable of producing IL-4, to the sites of inflammation*

The focus on IL-2 and IL-15 is also of interest given their functions[26]. The recruitment of T cells, NK cells and macrophages is a significant driver of localized immune response. Thus IL-16 and its suppression may facilitate localized tumor growth. As Hall et al have noted:

Interleukin-16 (IL-16) is reported to be a chemoattractant cytokine and modulator of T-cell activation, and has been proposed as a ligand for the co-receptor CD4. The secreted active form of IL-16 has been detected at sites of TH1-mediated inflammation, such as those seen in

[26] From Abbas et al, IL-2 performs the functions of T cells: proliferation and differentiation into effector and memory cells; promotes regulatory T cell development, survival, and function NK cells: proliferation, activation B cells: proliferation, antibody synthesis and IL-15 performs the function of NK cells: proliferation T cells: survival and proliferation of memory CD8+ cells. IL-4 functions as B cells: isotype switching to IgE T cells: Th2 differentiation, proliferation Macrophages: alternative activation and inhibition of IFN-γ–mediated classical activation.

autoimmune diseases, ischemic reperfusion injury (IRI), and tissue transplant rejection. Neutralization of IL-16 recruitment to its receptor, using an anti- IL16 antibody, has been shown to significantly attenuate inflammation and disease pathology in IRI, as well as in some autoimmune diseases.

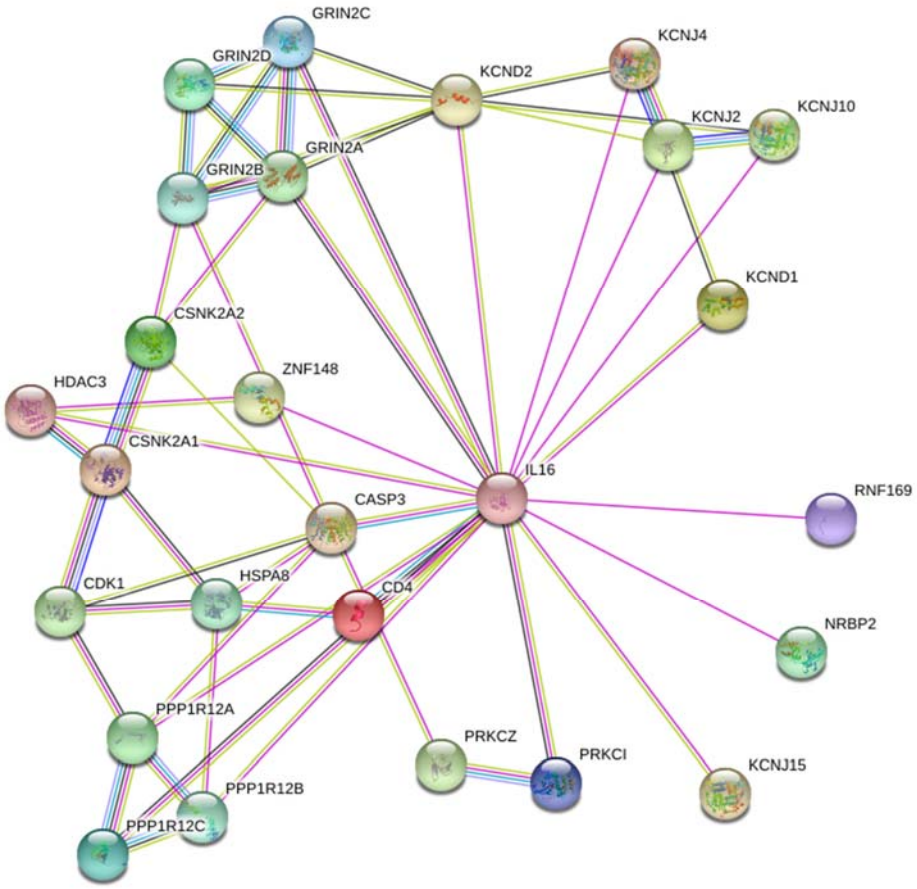
The 14.1 antibody is a monoclonal anti- IL-16 antibody, which when incubated with CD4cells is reported to cause a reduction in the TH1-type inflammatory response. Secreted IL-16 contains a characteristic PDZ domain. PDZ domains are typically characterized by a defined globular structure… In contrast to other reported PDZ domains, the solution structure previously reported for IL-16 reveals a tryptophan residue obscuring the recognition groove. We have solved the structure of the 14.1Fab fragment in complex with IL-16, revealing that binding of the antibody requires a conformational change in the IL-16 PDZ domain. …

Our study reveals a surprising mechanism of action for the antibody and identifies new opportunities for the development of IL-16- targeted therapeutics, including small molecules that mimic the interaction of the antibody.

The downregulation of IL-16 as a unique characteristic of FVPTC can be a significant factor. Yet as we have noted, the details of how this process functions and why appears to be missing. For example, if this were to be the case, then by simply increasing IL-16 directly in the tumor can we use that as an effective therapeutic?

We demonstrate the relationship map below[27]:

[27] https://version11.string-db.org/cgi/network.pl?taskId=SRBSp2hTdpOO

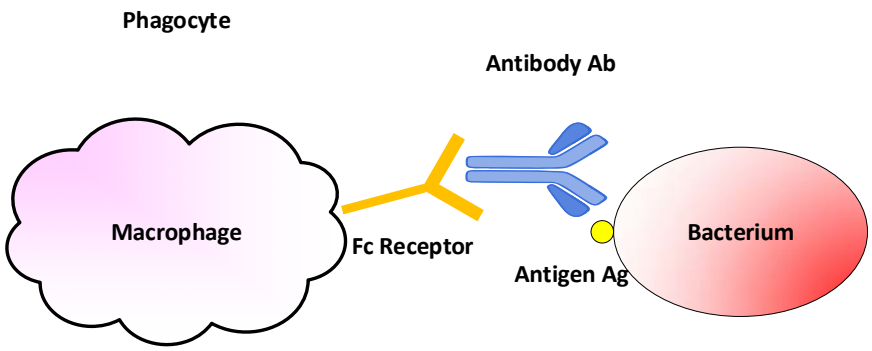


4.4 TCPTC

We now examine the TCPTC variant. We have previously seen this to be a more aggressive form. The impact here appears to be a result of immune associated gene changes.

4.4.1 FCGR

A fundamental paradigm for immune system attacks is shown below:



Namely we have a phagocyte with an Fc receptor attaching to an antibody attached to an antigen. This connection starts the process of attacking in this case the bacterium. The Fc receptor, FCR, is a key player in this attack category. The Fcϒ receptors or FCGR are most critical for phagocytosis.

Chakladar et al have stated:

We found most pathways dysregulated in TCPTC, including; TNF signaling; RXR/RAR pathways, and chemokine signaling, to be similar to those found in the two other PTC subtypes.

However, the IA pathways of ***FCGR dependent phagocytosis****; and* ***antigen processing and presentation*** *are uniquely dysregulated in TCPTC. The* ***FCGR-related genes are mostly downregulated and include key B-cell and antibody production-related genes, while antigen processing and presentation genes are mostly upregulated.*** *Because antibodies are produced against antigens, the dysregulation between the two pathways may be interrelated.*

They note above that FCGR is downregulated, thus inhibiting any phagocytosis based on an antigen presentation by a phagocyte. In contrast the genes controlling the antigen production/processing are increased.

As Dornan et al have noted:

The mechanisms of action by which monoclonal antibodies may have antitumor effects includes antibody-dependent cellular cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), and direct proapoptotic effects. ADCC is positively regulated by activating FCGRs expressed on natural killer (NK) cells, macrophages, and dendritic cells. A mouse model deficient for the activating FCGR3 locus has been shown to completely diminish the antitumor efficacy of monoclonal antibody therapies trastuzumab and rituximab.

Genomic polymorphisms of the FCGR3A and FCGR2A genes resulting in exchanges of amino acids, valine, or phenylalanine at position 158 of FCGR3A and histidine or arginine at position 131 of the FCGR2A, have been shown to influence the affinity of monoclonal antibodies to the FC-receptor on the effector cells. For FCGR3A the VV genotype at position 158 represents the high-affinity form while FV and FF genotype are associated with reduced affinity. For

FCGR2Athe HH genotype at position 131 represents the higher-affinity form, while HR and RR represent the lower-affinity form.

As Raab et al note regarding the impact on NK cells:

NK cells are cytotoxic lymphocytes and components of the innate immunity. They play a major role in antitumor immunity, and numerous attempts presently aim to employ NK cells for cancer therapy. Notably, this includes various strategies to induce ADCC by suitable antitumor Abs, as NK cells are recognized as a major cell population in humans that contributes to this important Ab function.

In general, NK reactivity is guided by a balance of activating and inhibitory signals far beyond the FcgRIIIa (CD16) (NOTE, this is also FCGR3A) that mediates ADCC. A prominent additional modulator of NK reactivity is the C-type lectin-like receptor NKG2D that potently induces antitumor immunity after recognition of its ligands (NKG2DL).

NKG2DL are generally absent on healthy cells, but induced upon cellular stress, including malignant transformation, and expressed on cancers of various origin, including breast cancer. In humans, the NKG2DL are comprised of MICA, MICB, and ULBP1-6. Due to the high prevalence of NKG2DL expression on malignant cells and their highly tumor-restricted expression pattern, we reasoned that NKG2DL constitute promising target Ags for an Ab-based immunotherapeutic strategy. In particular, such an approach would be applicable in breast cancer cases that lack HER2/neu overexpression.

Moreover, it needs to be considered that the expression pattern of the different NKG2DL varies largely among different tumor samples (14). Thus, we reasoned that, instead of employing a specific Ab targeting only a single NKG2DL (potentially not expressed in a high percentage of cancer cases), utilizing an NKG2D–Fc fusion protein that allows for binding to all NKG2DL constitutes a more promising approach, especially as techniques to increase the affinity of Fc parts to CD16 resulting in enhanced NK cell ADCC are available[28]*. We further reasoned that this would more than compensate for the loss of activating signals caused by binding of the NKG2D part of our constructs to its ligands and the resulting reduction of activating signals caused by disruption of NKG2D-NKG2DL interaction.*

Besides Fc-optimized NKG2D-Ig fusion proteins, constructs containing wildtype (WT) IgG1 or Fc parts with abrogated affinity to CD16 were used as controls. The ability of the (different) fusion proteins to modulate NK reactivity against breast cancer cells with varying levels of HER2/neu, alone or in combination with trastuzumab, was then preclinically characterized.

Our data indicate that Fc-optimized NKG2D-Ig constructs, by taking advantage of the tumor-associated expression of NKG2DL, constitute a promising immunotherapeutic strategy in particular for breast cancer cases with low or absent HER2/neu expression, in which trastuzumab treatment would be ineffective.

[28] Recall from Abbas: *Antibody-dependent cell-mediated cytotoxicity (ADCC) A process by which NK cells are targeted to IgG-coated cells, resulting in lysis of the antibody-coated cells. A specific receptor for the constant region of IgG, called FcγRIII (CD16), is expressed on the NK cell membrane and mediates binding to the IgG.*

The latter observation above is a viable means to deal with TCPTC. Perhaps it may also be an effective therapeutic in a broader context.

4.4.2 RAET

RAET can be described as follows:

The retinoic acid early transcript (RAET) proteins are stress-induced ligands for the immune cell activating receptor NKG2D. While the RAET ligands can signal immune recognition of cancer cells, it is likely that immunosuppression within the microenvironment prevents effective RAET signaling in TCPTC, and the upregulation of NKG2D ligands have been linked to poor prognoses in other cancers.

Specifically, RAET1E and RAET1G are upregulated. Both are receptors for NKG2D as we note below. Thus, we focus on NKG2D as the prime genetic target.

4.4.3 NKG2D

NKG2D is a receptor found on CTLs. As Abbas et al have noted:

Tumor cells become susceptible to killing by NK cells when they down-regulate expression of class I MHC or they upregulate expression of ligands that bind activating NK cell receptors. NK cells express inhibitory receptors that bind class I MHC molecules expressed on healthy cells. As we will see later, some tumors lose expression of class I MHC molecules, as a result of selection against class I MHC–expressing cells that are readily killed by CTLs. This loss of class I MHC molecules makes the tumors particularly good targets for NK cells.

In addition, many tumors express ligands for the NKG2D activating receptor on NK cells, such as MIC-A, MIC-B, and ULB, and NKG2D signaling can override inhibitory signals from class I MHC binding receptors. NK cells may also be activated to kill tumor cells coated with antitumor antibodies by antibody dependent cell-mediated cytotoxicity*. The tumoricidal capacity of NK cells is increased by cytokines, including interleukin-2 (IL-2), IL-15, and IL-12, and the antitumor effects of these cytokines in vivo are partly attributable to stimulation of NK cell activity.*

Thus, NKG2D can be a significant player in immune responses. The authors Chakladar et al, have noted:

*only the expressions of **RAET1E and RAET1G** correlated with clinical variables—specifically pathologic stage. The retinoic acid early transcript (RAET) proteins are **stress-induced ligands for the immune cell activating receptor NKG2D** While the RAET ligands can signal immune recognition of cancer cells, it is likely that immunosuppression within the microenvironment prevents effective RAET signaling in TCPTC, and the upregulation of NKG2D ligands have been linked to poor prognoses in other cancers*

From Lam et al:

The NKG2D system is an arm of innate immune recognition, which is important in the context of both cancer and infection. Transformed and infected cells increase their expression of NKG2D ligands (NKG2DL). Engagement of the NKG2D receptor on natural killer (NK) cells and certain T cells stimulates their effector functions, which aid in tumor control. Recently, we elucidated a principle mechanism that induces NKG2DLs in cancer cells: the DNA damage response (DDR).

DNA damage upregulates the expression of numerous NKG2DLs, including different retinoic acid early transcript (RAE1) isoforms and mouse UL16-binding protein-like transcript 1 (MULT1) in mouse cells. The DDR molecules ataxia telangiectasia and Rad3 related (ATR) ataxia telangiectasia mutated homolog (ATM), and checkpoint kinase 1 homolog (CHK1) are required for expression of NKG2DLs in response to DNA damage and the constitutive expression of NKG2DLs in some tumor cell lines. Additional effector molecules of the DDR required for mouse NKG2DL expression have not been identified.

Likewise, Lanier notes:

NKG2D is an activating receptor expressed on the surface of natural killer (NK) cells, CD8+ T cells, and subsets of CD4+ T cells, invariant NKT cells (iNKT), and y8 T cells. *In humans, NKG2D transmits signals by its association with the DAP10 adapter subunit, and in mice alternatively spliced isoforms transmit signals either using DAP10 or DAP12 adapter subunits. Although NKG2D is encoded by a highly conserved gene (KLRK1) with limited polymorphism, the receptor recognizes an extensive* ***repertoire of ligands,*** *encoded by* ***at least eight genes in humans (MICA, MICB, RAET1E, RAET1G, RAET1H, RAET1I, RAET1L, and RAET1N),*** *some with extensive allelic polymorphism.*

Expression of the NKG2D ligands is tightly regulated at the level of transcription, translation, and post- translation. In general, healthy adult tissues do not express NKG2D glycoproteins on the cell surface, but these ligands can be induced by hyperproliferation and transformation, as well as when cells are infected by pathogens. Thus, the NKG2D pathway serves as a mechanism for the immune system to detect and eliminate cells that have undergone "stress."

Viruses and tumor cells have devised numerous strategies to evade detection by the NKG2D surveillance system, and diversification of the NKG2D ligand genes likely has been driven by selective pressures imposed by pathogens*. NKG2D provides an attractive target for therapeutics in the treatment of infectious diseases, cancer, and autoimmune diseases*

Finally, as Vivier et al have noted:

Besides using inhibitory receptors that recognize self, NK cells are also equipped with cell surface activating receptors. In addition to the recognition of microbial molecules by a variety of innate immune receptors, the so-called 'infectious non-self-recognition', it has been shown that several receptors of innate immune cells can detect internal changes that occur in damaged host tissues, leading to the concept of 'stress-induced' self-recognition". This mode of detection relies on the recognition of self-molecules that are barely detectable in steady-state conditions, but whose expression increases in various forms of stress.

A prototypical example is the activation of NK cells via engagement of the activating NKG2D receptor. NKG2D interacts with self-molecules that are selectively up-regulated on stressed cells, such as tumour cells. ***In vivo, NKG2D was shown to be crucial for immunosurveillance of epithelial and lymphoid malignancies in two transgenic models of de novo tumour genesis.***

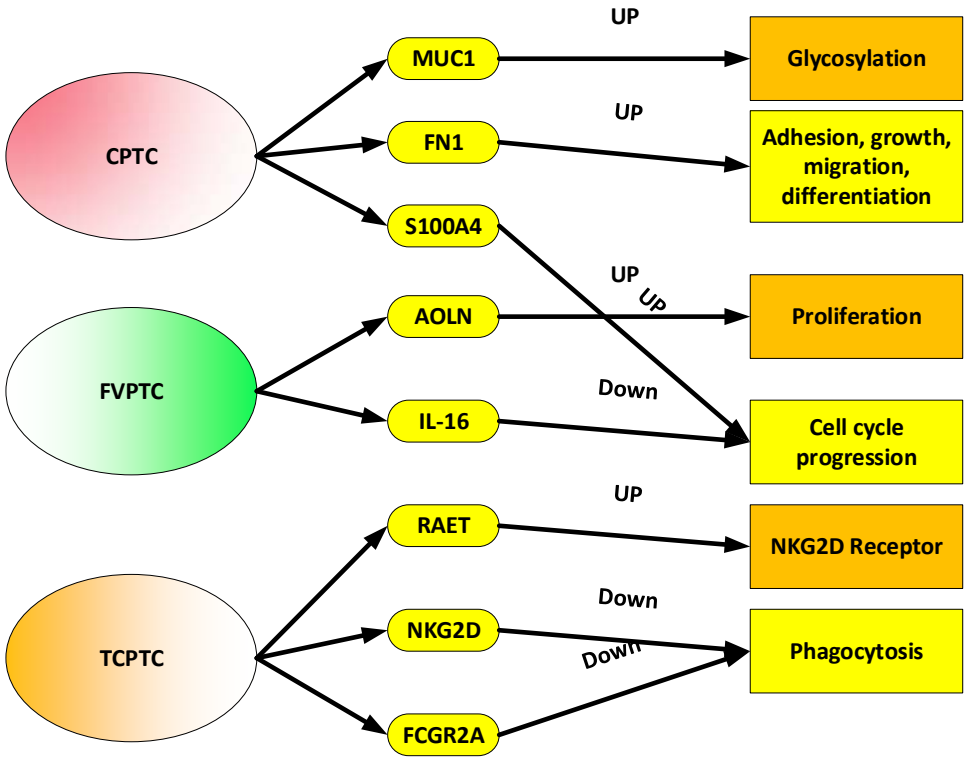
In the transgenic Eμ-Myc mouse model of spontaneous B cell lymphoma, the tumour expression of NKG2D ligands represents an early step of tumour genesis that is associated with still unknown genetic lesions of cancer cells. NKG2D ligands are the stress-inducible MICA/B and ULBP/RAE proteins in humans and Rae1, H60, and Mult-1 proteins in mice. A link between tumour genesis, DNA damage response (DDR) and the immune response has been proposed.

DNA-damaging agents or DNA lesions associated with tumour genesis activate the DDR, which results in up-regulation of NKG2D ligands leading NK cells to attack the diseased cells. The up-regulation of NKG2D ligands depends on the PI-3 kinase-related ATM (ataxia telangiectasia, mutated) or ATR (ATM- and Rad3-related) protein kinases, which initiate the DDR pathway after exposure to DNA damage 73. Treatment with proteasome inhibitors also induces NKG2D ligand expression in multiple myeloma cells via the ATM/ATR pathway.

Thus, the change in NKG2D can be a significant marker especially in the TCPTC. It also is the interaction between the RAET and NKG2D which is of major concern.

4.5 SUMMARY

We have combined the above into the figure below where we have listed the genetic factors observed. Then on one side we delineate their functions or actions and on the other side link them to the specific PTC



The analysis of the above may have some usefulness. It is suspected that a paradigm as the above could be a useful therapeutic tool, linking class, gene, and process.

5 OBSERVATIONS

We now consider several observations resulting from an examination of this work by Chakladar et al and its application to PTC and its variants.

5.1 WERE THE GENES SO NOTED DISPOSITIVE OR JUST OPPORTUNISTIC?

There is a fundamental question as to where and why the genes were selected. Were they selected merely opportunistically, namely this is what happened? Or were they dispositive as we tried to analyze herein. The authors clearly performed a substantial and exhaustive analysis. Yet the question we frequently pose is: are these the genes which cause the problems? Or are they merely free riders in the process, perhaps resulting from yet to be ascertained genes or processes?

The key question should be: are the genes identified ones whose resulting effects are such that the malignant behavior of the cells is a result? Namely, is the gene a proliferation gene gone wild, or is the gene one which facilitates uncontrolled cellular structure, or is the gene one which inhibits immune responses which would normally have eliminated the aberrant cells? One should be cautious in just displaying a set of genes which are present without following through on these or similar questions.

5.2 WHAT ARE THE RESULTS IN CELLULAR STRUCTURE FROM USING THE GENE PROFILES?

Histology reflects structure as we observe it with tools available. We have recently addressed this issue in a similar vein[29]. In this previous work we asked the question; how do genes determine cellular, nuclear and nucleolar structure and form which the histology expresses? That seems to be the essential question to all we are discussing herein. However. there does not seem to be a dispositive answer to this at the present time.

For example, we have no genetic understanding of why tall cells occur, why Orphan Annie eyes occur and so forth. But based upon discussions herein, with PTC we do have MUC1 and FN1 on CPTC which appear to be logical choices for the development of papilla. It would be of interest to analyze the physics of such formations as one sees changes in the adhesion characteristics. We do see some of these types of effects in nano particle assemblages.

5.3 WHAT OF EPIGENETIC FACTORS?

All the genes we observed are arguably reasonable. But what of the epigenetic factors? What of miRNAs and methylation and acetylation and the like. How do we include them in our overall analyses of the classification problem. We have argued elsewhere that miRNAs can have a significant effect as well as the effects of methylation[30].

[29] https://www.researchgate.net/publication/334429457_miRNAs_Genes_and_Cancer_Cytology

[30] https://www.researchgate.net/publication/334429457_miRNAs_Genes_and_Cancer_Cytology

5.4 WHAT IS TRUTH: GENES, HISTOLOGY, NEITHER, BOTH?

The seems to be between genetic profiles and histological readings. For the past century as the histologist has fine-tuned their tools and means to ascertain what cells individually and collectively look like, the genetic analysis has progressed. In the case discussed herein, we have an interesting examination of which one do we prefer to diagnose what. Does classic histology now take a back seat to the genetic profile or do we really know enough of the genetic profile to use it reliably. One example is that in prostate cancer there is a building consensus that there exist stem cells, and that even with cell by cell sequencing we may not have done so with the stem cell which is one in a million. Thus, with the tools currently available we must rely upon the tried and true methods of histology. But when we can reliably, cost effectively, and in a timely manner sample all cells in a sample then we may be able to have profiles which diagnose, prognose and determine the best therapeutic. The question is; how close are we here?

5.5 WHAT ARE THE CLINICAL IMPLICATIONS?

Now Chakladar et al note in their abstract the following:

However, unique cancer or immune-associated genes are associated with clinical variables for each subtype. Cancer-related genes MUC1, FN1, and S100-family members were the most ***clinically relevant*** *in CPTC, while APLN and IL16, both immune-related, were* ***clinically relevant*** *in FVPTC. RAET-family members, also immune-related, were* ***clinically relevant*** *in TCPTC. Collectively, our data suggest that dysregulation of both cancer and immune associated genes defines the gene expression landscapes of PTC variants, but different cancer or immune related genes* ***may drive the phenotype*** *of each variant.*

Upon extensive readings the elucidation of what those "clinically relevant" determinations were in TCPTC, FVPTC and CPTC. As we noted in the introduction, the histology (morphology), the genomic structures and alterations may lend understanding to the prognosis and in turn treatment. The latter two are the measures of clinical relevance.

5.6 FNA VERSUS SINGLE CELL POST RESECTION GENOMICS

Genomic testing is performed on FNA results. Also, sometimes genomic testing is done on single cell extractions from a thyroidectomy or lobectomy. How comparable are the results? Are they diagnostic and if so how do they compare to the histological studies?

5.7 IS IT REASONABLE TO ASSUME THAT GENOMIC CLUSTERING CAN REPRODUCE PTC CLASSIFICATIONS COMPARABLE TO CURRENT HISTOLOGICAL RESULTS?

Genomic clustering can be performed here. In a sense it is clustering with learning if one were to employ the histological reference point. However, if one were to use all the genomic data and then cluster with it one could eliminate common genomic metrics and derive the clustering metrics as may be determined. Admittedly this may be a complex process. The downside again is the issue of stem cell versus the other putatively malignant cells.

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