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Molecular Genetics and Cellular Characteristics of *TFE3* and *TFEB* Translocation Renal Cell Carcinomas

Eric C. Kauffman[1], **Christopher J. Ricketts**[1], **Soroush Rais-Bahrami**[1], **Youfeng Yang**[1], **Maria Merino**[2], **Donald Bottaro**[1], **Ramaprasad Srinivasan**[1], and **W. Marston Linehan**[1]

[1]Urologic Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

[2]Department of Pathology, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

Abstract

Despite nearly two decades since the discovery of gene fusions involving *TFE3* or *TFEB* ("*TFE*") in sporadic renal cell carcinoma (RCC), the molecular mechanisms underlying the renal-specific tumorigenesis remains largely unclear. The recent publication of the TCGA Network's clear cell kidney cancer paper provides further evidence for the importance of gene fusions by identifying 5 tumors harboring *SFPQ-TFE3* fusions that otherwise lacked the common clear cell RCC associated mutation. Herein, we review key molecular features of *TFE*-fusion RCC, including candidate signaling pathways contributing to oncogenesis and a detailed overview of gene fusion isoforms based on an updated knowledge of *TFE* genetic organization. A total of 5 *TFE3* gene fusions (*PRCC-TFE3*, *ASPSCR1-TFE3*, *SFPQ-TFE3*, *NONO-TFE3*, *CLTC-TFE3*) and 1 *TFEB* gene fusion (*MALAT1-TFEB*) have been identified in RCC tumors and characterized at the mRNA transcript level, with considerable heterogeneity in exon structure across different tumors, even for the same fusion partners. Common to all *TFE3* and *TFEB* fusion isoforms is the retention of the wild-type protein C-terminus, including domains for DNA-binding, dimerization, and nuclear localization, but interestingly, not transcriptional activation. Despite this, the most widely accepted model explaining *TFE*-fusion oncogenesis remains the introduction of a constitutively active promoter leading to dysregulated *TFE* transcriptional activity. A multitude of molecular pathways well-implicated in carcinogenesis are regulated in part by TFE3 and/or TFEB protein, including activation of TGFβ and ETS transcription factors, E-cadherin expression, CD40L-dependent lymphocyte activation, mTORC1 signaling, insulin-dependent metabolism regulation, folliculin signaling, and Rb-dependent cell cycle arrest. Determining which pathways are most important will be critical to discovering the most promising therapeutic targets for this disease. Useful to this goal is a panel of cell lines derived from different *TFE3*-fusion RCC patient tumors, representing multiple fusion isoforms.

1 Introduction

Chromosomal rearrangement resulting in the fusion of two different genes is the most common type of mutation in human cancer.[1] The role of these mutations in sarcomas and hematologic malignancies has been well established for several decades but has only recently become more apparent in common carcinomas due to advances in genetic analysis.[2]

(Figure 1) The Next-generation sequencing of breast and prostate carcinomas has highlighted the common nature of this event in carcinogenesis by demonstrating numerous gene fusions per patient tumor.[2-4] Although the effect and importance of all these gene fusions has yet to be fully elucidated, it underscores the fact that they represent a common mutation event.

Renal cell carcinomas (RCC) include multiple heterogeneous cancer types originating from renal tubular epithelium.[5] Each is defined by a distinct histology, the most common types including clear cell (65-70%), papillary (15-20%), and chromophobe (5-10%).[6] Genetic mutations underlying RCC tumorigenesis are increasingly understood, aided by the discovery of least 12 genes (*VHL*, *MET*, *FH*, *FLCN*, *SDHB*, *SDHC*, *SDHD*, *TSC1*, *TSC2*, *PTEN*, *MiTF* and *BAP1*) whose germline mutation confers hereditary RCC susceptibility.[7,8] Additional insight has come from recent full-exome sequencing efforts in sporadic clear cell RCC tumors including the Cancer Genome Atlas (TCGA) Network's clear cell kidney cancer project (KIRC). These have revealed the importance of mutations within chromatin remodeling genes, including *PBRM1*, *SETD2*, *KDM6A* (*UTX*), *KDM5C* (*JARID1C*), ARID1A and *BAP1*, as well as confirming the continued importance of *VHL* mutation and chromosome 3p loss.[9-11]

Gene fusions of two genes from the micropthalmia transcription factor (MiT) gene family genetically define a histologically variable group of approximately 1-5% of sporadic RCC tumors.[12-14] The MiT family includes four structurally related genes (*TFE3*, *TFEB*, *TFEC*, and *MiTF*), which regulate a variety of tissue-specific functions contributing to cell differentiation. (Table 1, Figure 2) While one member of the family, *MiTF*, has been associated with hereditary RCC susceptibility; two other members, *TFE3* and less commonly *TFEB*, are found fused to other genes in sporadic RCC tumors that are often referred to as “translocation RCC”, although chromosomal rearrangements other than translocations can also cause these fusions. In contrast to the histologically defined RCC types, these gene fusion associated kidney cancers are more common among pediatric patients, with the prevalence peaking in early adulthood.[15,16] Clinical behavior of RCC with the *TFE3* fusion is relatively aggressive, with metastasis common at presentation. Yet despite nearly two decades since the discovery of the MiT family gene fusions in RCC, the molecular biology underlying these cancers remains largely uncharacterized and effective targeted therapies are yet to be identified. Hence, there remains no clinical standard available for pharmacologic treatment of these patients.[15]

Critical to developing effective therapeutics for RCC patients with *TFE3* or *TFEB* gene fusions is identification of the key clinical pathways driving these particular cancers. Herein, we summarize the contemporary understanding of the molecular biology underlying these gene fusion associated (translocation) RCCs, which we subsequently refer to within as “*TFE*-fusion RCCs”. Our report includes an up-to-date overview of the candidate mechanisms and signaling pathways thought to contribute to oncogenesis, as well as a detailed description of gene fusion structures based on an updated understanding of *TFE3* and *TFEB* genetic organization. Clinical traits and management recommendations for *TFE*-fusion RCC patients are reviewed in detail elsewhere.[15]

2 Multiple TFE gene fusion partners: a historical perspective

2.1 Transcription factor binding to IGHM enhancer 3 (TFE3)

The first documented case of an Xp11 rearrangement in an RCC tumor was described over 25 years ago as a t(X;1)(p11.2;q21.2) translocation in a pediatric patient.[17] Follow-up reports revealed this translocation to be a recurrent mutation in adults.[18,19] At this time, three immortalized cell lines (UOK120, UOK124, and UOK146) derived from RCC patient tumors with papillary-like histology and the t(X;1)(p11.2;q21.2) mutation were established in our laboratory, and their study led to the cloning and description of the first gene fusion in RCC, *PRCC-TFE3*, in 1995.[20-23] This fusion was noted to link the gene encoding a previously described transcription factor, *Transcription factor binding to IGHM enhancer 3* (*TFE3*), on chromosome Xp11.2 with a novel gene on chromosome 1q21.2, designated *Papillary renal cell carcinoma (translocation-associated)* or *PRCC* at the time due to its presumed importance in papillary RCC oncogenesis. This discovery marked the first gene fusion identified in a carcinoma other than thyroid cancer.

Subsequently, two other RCC patient cell lines were created within our laboratory (UOK145 and UOK109) harboring two novel Xp11.2 rearrangements, t(X;1)(p11.2;1p34) and inv(X)(p11.2;q12), from which we cloned and described two additional gene fusions, *SFPQ-TFE3* (previously referred to as *PSF-TFE3*) and *NONO-TFE3* (previously referred to as *p54nrb-TFE3*), respectively.[24] This work underscored that *TFE3*, rather than *PRCC*, was the critical gene fusion partner. Intriguingly, both the *Splicing factor proline/glutamine-rich* (*SFPQ*) and *Non-POU domain containing, octamer-binding* (*NONO*) genes were noted at this time to encode functionally conserved mRNA-splicing proteins, possibly implicating this process in *TFE3*-fusion oncogenesis or that nuclear localization was important. Characterization of the *NONO-TFE3* fusion, resulting from inversion of the *TFE3* and *NONO* loci on chromosome Xp, revealed that chromosomal rearrangements other than translocations could generate *TFE3* gene fusions.

In 2001 and 2003, Argani *et al* identified the 4th and 5th *TFE3* fusions in RCC, *ASPSCR1-TFE3* (previously referred to as *ASPL-TFE3*) and *CLTC-TFE3*, respectively.[25,26] Both the *Alveolar soft part sarcoma chromosome region, candidate 1* (*ASPSCR1*) and *Clathrin - heavy chain* (*CLTC*) were found to localize to chromosome 17q. The former was cloned as a novel gene of unknown function and remains as such to date, while the latter had been previously cloned as the heavy chain component of the vesicular transport protein, clathrin.[25,26]

A total of five partner genes have thus been identified in fusions with *TFE3*. (Table 2) Only three of these five *TFE3* gene fusions (*PRCC-TFE3*, *ASPL-TFE3*, and *PSF-TFE3*) are confirmed as recurrent mutations in multiple RCC patients with differing gene fusion boundaries being observed and designated as “types”, while *NONO-TFE3* and *CLTC-TFE3* have thus far each been identified in only a single patient. *TFE3* gene fusions are not unique to RCC patients. The *ASPSCR1-TFE3* fusion was originally identified in 100% of alveolar soft part sarcomas (ASPS), a rare lung cancer variant with no known RCC association, and the *SFPQ-TFE3* fusion and possibly other *TFE3*-fusions are also found in a subset of rare tumors known as perivascular epithelioid cell neoplasms (PEComas).[27,28] The existence of

additional *TFE3*-fusion partners is suggested by case reports of Xp11.2 rearrangements with other chromosomal loci in RCC tumors, including 3q23 and 19q13.1, however *TFE3* fusions were not genetically confirmed in these studies.[29,30]

The recent publication of the TCGA Network's clear cell kidney cancer project (KIRC) paper provides further evidence for the importance of gene fusions in RCC and identified 5 tumors harboring *SFPQ-TFE3* fusions by RNASeq analysis.[11] These newly identified *TFE*-fusion RCCs provided both a new type of *SFPQ-TFE3* fusion, with gene boundaries including more of the *TFE3* gene than any previous fusion (Table 2), and the most intense genetic analysis of a *TFE*-fusion RCC. Exome sequencing analysis demonstrated that only one of these tumors had a VHL mutation, normally very common in cell clear RCC, and no tumors demonstrated mutations in any of the commonly mutated genes in clear cell RCC (*PBRM1*, *SETD2*, *BAP1*, *MTOR*, *PIK3CA*, *ARID1A*, *ATM*, *PTEN*, *KDM5C*).[11] This lack of mutation in RCC associated genes strongly suggests that the TFE-fusions are the driving force in these tumors without the necessity for common associated mutations.

2.2 Transcription factor EB (TFEB)

Chromosomal rearrangements resulting in the fusion between the *Transcription factor EB* (*TFEB*) gene on chromosome 6p21.2 and the non-protein encoding *Metastasis associated lung adenocarcinoma transcript 1* gene known as *MALAT1* (previously referred to as *Alpha*) on chromosome 11q13 is the most recently described gene fusion in RCC.[31,32] Its discovery provided the initial evidence of a common role for the *MiT* family in kidney cancer. Less than two dozen cases are documented with genetic confirmation of the *MALAT1-TFEB* fusion or t(6;11)(p21.2;q13) mutation to date, and the incidence of this fusion is approximately 1:15-1:20 compared to that of *TFE3* fusions.[15] No cell line has yet to be derived from *TFEB*-fusion RCC patients. In contrast to the multiple fusion partners of *TFE3*, no *TFEB* fusion partners other than *MALAT1* are presently known, although a t(6;17)(p21;q24-25) translocation reported in a pediatric RCC tumor has suggested a potential novel *TFEB* fusion partner on chromosome 17q24-25, the same locus of the *ASPSCR1* gene found in *TFE3* fusions.[33] Also in contrast to TFE3 fusions, the *MALAT1-TFEB* fusion may be associated with a favorable clinical prognosis.[15]

3 TFE Gene Fusion Structure and mRNA Transcript Isoforms

The precise nucleic acid site of the fusion between *TFE3 or TFEB* and their respective fusion partners varies and can produce fusions containing differing number of exons in the case of *TFE3* and its gene partners. Accordingly, different mRNA transcript isoforms have been characterized for most known *TFE* fusions and have been designated with a numbered “type”. (Figure 3) Unfortunately, the published literature invokes some confusion regarding the structure of chimeric *TFE3* transcripts, due to inconsistencies in exon nomenclature following the recent identification of two additional upstream coding exons and a novel transcriptional start site. Here we provide a summary of different *TFE* fusion transcript isoform structures characterized to date, based on comparison of sequence data from the *TFE*-fusion literature and contemporary genetic databases, including the UCSC Genome Browser on Human (February 2009; GRCh37/hg19; http://genome.ucsc.edu), the Ensembl

database (release 69 - October 2012; http://www.ensembl.org/) and the UniProt Protein Database (November 2012; http://www.uniprot.org). (Table 2, Figure 2)

3.1 Wild-type TFE3

The *TFE3* gene (Figure 3A) is encoded by 14,749 base pairs (bp) on chromosome Xp11.22 and produces a 3,431-bp mRNA transcript consisting of a 238-bp 5′ untranslated region (UTR) followed by ten coding exons and a 1,427-bp 3′ UTR (NM_006521.4). The coding region includes a start codon in exon 1 and stop codon in exon 10. Wild-type *TFE3* mRNA is translated into a 575 amino-acid (AA) protein (61.5 kD) sharing highly conserved peptide domains with other MiT family members, including a 12-AA transcription *activation domain* (AD 260AA-271AA) spanning exons 4-5, a 54-AA *basic region* and *helix-loop-helix* domain (bHLH 346AA-399AA) within exons 7-9, and a 22-AA *leucine-zipper domain* (LZ 409AA-430AA) within exons 9-10. (Figure 2, Figure 3A) The bHLH and LZ domains mediate dimerization and DNA binding,[34,35] and the former additionally contains a putative nuclear localization signal (NLS).

3.2 TFE3 gene fusions

The exact breakpoint site in *TFE3* fusions varies and is presumed to occur with different introns of both the *TFE3* gene and its fusion partners. Pre-mRNA splicing of the gene fusion generates a chimeric mRNA transcript fused at exon-exon junctions that maintains a coding open reading frame that reads from one partner gene to the other and it is these transcripts that have been successfully mapped to define the gene fusion types. The chimeric mRNA transcripts encode the N-terminal portion of each fusion partner linked to a range of the C-terminal encoding exons of *TFE3*, including the recently identified *SFPQ-TFE3* type 3 fusion that contains nearly the entirety of *TFE3* (exons 2-10). (Figure 3B-F) The *TFE3* exon 1 is invariably absent (reports prior to 2004 of exon 1 in chimeric transcripts refer to exon 3 using updated nomenclature)and the universally retained region of the *TFE3* gene (exons 6-10) corresponds to a 280-AA C-terminal peptide, which includes the bHLH/LZ dimerization/DNA-binding domains and the putative NLS, but not all of the strong transcription activation domain.[21,23-26,36-38] The *TFE3-PRCC* fusion has the greatest degree of transcript heterogeneity, with at least four isoforms characterized to date.[21,23,36] The reciprocal transcript encoding the *TFE3* N-terminus fused to the fusion partner C-terminus is not consistently generated, and is thus generally believed to be unimportant in *TFE3*-fusion oncogenesis.[21,23,25] TFE3 fusion proteins vary considerably in size, from the smallest *PRCC-TFE3* isoform (type 4) at 452 AA (47.8kDa) to the largest *CLTC-TFE3* at 1,212 AA (136.1 kDa), more than twice the size of the wild-type protein. (Table 2, Figure 3) As described above, a series of cell lines have been derived from the tumors of *TFE3*-fusion RCC patients that represent most of the different gene fusions, including multiple *PRCC-TFE3* isoforms. (Table 2)

3.3 Wild-type TFEB

The *TFEB* gene is encoded by 51,083 bp on chromosome 6p21.1. The gene encodes a 2,364bp mRNA transcript consisting of two non-coding and eight coding exons, with a 302-bp 5′ UTR followed by a start codon in exon 3, and a stop codon in exon 10 followed by a

621bp 3′ UTR (NM_007162.2). (Figure 4) A variant transcript isoform contains only one alternative non-coding exon (NM_001167827.1). Wild-type *TFEB* mRNA produces a 476-AA protein similar to TFE3 with a 10-AA strong transcription *activation domain* (AD 156AA-165AA), a 54-AA *basic region* and *helix-loop-helix* domain (bHLH 235AA-288AA) containing a putative *nuclear localization signal* (NLS) and a 22-AA *leucine-zipper* DNA-binding domain (LZ 298AA-319AA). (Figures 2, Figure 4)

3.4 TFEB gene fusions

MALAT1-TFEB fusion breakpoints generally occur before the start codon in *TFEB* exon 3, resulting in retention of the complete *TFEB* coding sequence. (Figure 4) Until recently, all *MALAT1-TFEB* fusions were believed to occur within a 289-bp breakpoint cluster region (BCR) upstream of *TFEB* exon 3, and within a 1,205-bp BCR of *MALAT1*.[31,32,39-42] (Figure 4) However, a recent report by Inamura *et al* of three new cases of *TFEB*-fusion RCC has suggested a wider range of the traditional BCRs in both *TFEB* and *MALAT1*.[43] This study also suggested that a breakpoint can occur in *TFEB* exon 4, after the start codon, although the protein product produced was shown to be a similar size to the wild-type protein.[43] (Figure 4)

4 Developmental Roles of MiT Transcription Factors

TFE3 and *TFEB* are members of the MiT transcription factor family, which also includes *TFEC* and the prototype member, *MiTF*. MiT transcription factors form homo- or heterodimers that bind target promoters at a consensus E-box sequence motif (CA[C/T]GTG). Members have overlapping transcriptional target specificity due to highly conserved bHLH and LZ domains.[35,44] MiT functions are diverse and tissue-specific, often related to cell growth and differentiation. With the exception of *TFEC*, all MiT members have ubiquitous tissue expression, although activity is still tightly regulated in a tissue-specific manner.[45-49] This may occur in part through tissue-specific splicing, which in turn may be mediated by promoter multiplicity.[50,51] Additionally, *TFEC* inclusion can repress transcriptional activation by its family members in heterodimers, adding another potential level of regulation complexity.[52]

The most well characterized function of the MiT family is its master regulatory role in melanocyte differentiation, best described for *MiTF*. Over a dozen rodent models with germline *MiTF* mutations in the bHLH-LZ regions are described with various pigmentation defects including albinism, ocular defects (micropthalmia), and deafness.[35,53-58] In humans, heterozygous *MiTF* mutations are responsible for Waardenburg Syndrome IIA, characterized by melanocyte deficiency and similar phenotypic manifestation. Melanocytic differentiation appears to be mediated by MiT transcriptional activation of key melanocytic enzymes, tyrosinase and tyrosinase-related proteins (TRP)-1 and -2.[59,60] Important in this role may also be the ability of MiT members to bind and enhance activity of the LEF-1 protein, a mediator of the Wnt signaling pathway.[61]

Another well-described role of the MiT family is the regulation of hematopeotic cell differentiation, including for macrophages, osteoclasts, lymphocytes, and mast cells.[53,62-66] Phosphorylation of either TFE3 or MiTF by p38 MAP kinase in response to

osteoclastogenic cytokines, macrophage colony-stimulating factor (M-CSF), and receptor activator of nuclear factor kappa-B ligand (RANKL) is required for osteoclast differentiation.[67,68] Several osteoclast-related genes targeted by MiT transcription factors may play a role, including CLCN7, cathepsin K, Oscar, Ostm1, and TRAP.[62,69-71] Importantly, either MiTF or TFE3 alone is sufficient to activate osteoclastogenesis, underscoring the phenomenon of functional redundancy within the MiT family, with one member able to rescue loss of another.[35,62,72,73] Certain MiT gene mutations may act as a "dominant negative" if they inhibit protein function but allow continued heterodimerization. Hence, MiTF mutations in the basic domain lead to osteoporosis in mice despite wild-type TFE3, presumably due to TFE3 sequestration in transcriptionally inactive heterodimers with the mutant MiTF protein.

5 Mechanism of TFE-Fusion Oncogenesis: Dysregulated Protein Activity

Oncogenic activity of *TFE3* gene fusions has been demonstrated in various preclinical cancer models. For example, ectopic expression of the *PRCC-TFE3* fusion confers tumorigenicity to fibroblast cells in nude mice and enables benign proximal renal tubule cells to overcome *in vitro* growth arrest.[74] Similarly, expression of the *NONO-TFE3* fusion is required for soft-agar colonization by the UOK109 *TFE3*-fusion RCC cell line.[72] However, the molecular mechanisms behind *TFE*-fusion renal oncogenesis remain poorly understood. Three different models can be proposed to explain the oncogenic behavior of gene fusions in general. The first model ("lost activity model") presumes a wild-type tumor-suppressive gene function which is disrupted by the gene fusion; however, a fusion protein is not generally translated in such cases. The second model ("novel activity model") involves a novel transforming activity in the fusion protein due to altered protein conformation. In the third model ("dysregulated activity model"), which is the most widely accepted for *TFE3* gene fusions, the fusion protein upregulates oncogenic activity already present in the wild-type protein through introduction of a more active or less tightly controlled transcriptional promoter that is not regulated in a similar manner to wild-type *TFE3* promoter. Consistent with this mechanism, all TFE3 fusion partners have constitutively active gene promoters, and TFE3 fusion proteins are expressed at dramatically higher levels than wild-type TFE3.[23,24,26,32,74-76] Furthermore, the dysregulated activity model is known to be the mechanism for *TFEB*-fusion oncogenesis, with *MALAT1-TFEB* fusions generally upregulating a full-length wild-type TFEB protein as opposed to a chimeric protein. The dysregulated activity model is consistent with gene fusion mechanisms in other cancers, in which promoter substitution leads to upregulation of a transcription factor oncogene, such as *TMPRSS2-ERG* in prostate cancer. (Figure 1)

The dysregulated activity model suggests therefore that function of the wild-type *TFE3* and *TFEB* proteins (and perhaps other MiT family members), irrelevant of the gene fusion mutation, is at least in part pro-oncogenic and the MiT family members oncogenic activity has in fact been described in various cancer types.[72,77] In a preclinical model of clear cell sarcoma (CSS), for example, wild-type TFE3 protein and wild-type MiTF protein can both mediate the mitogenic activity of the pathognomonic *EWS-ATF1* oncoprotein.[72] While in UOK109 RCC cells, wild-type MiTF can rescue soft-agar colonization inhibited by endogenous *NONO-TFE3* knockdown.[72] Wild-type TFEB similarly has mitogenic effects in

some cancer types.[77] The oncogenic activity of MiT members is also supported by clinical observations. For example, *MiTF* is amplified in 20% of metastatic melanomas, and a germline activating *MiTF* amino-acid substitution recently been identified in association with increased risk of RCC and/or melanoma.[78-80] (Table 1) In RCC patients, higher TFE3 expression correlates independently with worse patient outcomes.[81,82]

6 Candidate Dysregulated TFE Signaling Pathways

6.1 Diversity of TFE signaling pathways

A variety of signaling pathways are employed by *TFE3* and *TFEB*, the dysregulation of which might contribute to renal carcinogenesis. (Figure 5) TFE3 is known to cooperate with SMAD proteins in mediating the TGFβ signaling pathway, which has a well-characterized role in carcinogenesis regulation. Important to this mechanism may be transcriptional activation of the *plasminogen activator inhibitor 1* (*PAI1*) gene, a regulator of fibrinolysis implicated in the metastasis of many cancer types.[83,84] TFE3 also directly binds and enhances activity of ETS-1, the prototype member of the ETS transcription factor family found in gene fusions among prostate cancer and sarcomas.[85] TFE3 and TFEB additionally govern transcription of E-cadherin, an important regulator of cancer cell-cell interactions, as well as CD40L, the primary activator of T-cell lymphocytes, raising a possible role in tumor immunoevasion.[73,86]

Both TFE3 and particularly TFEB have also been implicated in mTORC1 signaling, a major regulator of protein synthesis contributing to tumor growth for many cancer types, including RCC.[87] Argani *et al* reported mTORC1 activation to be more common among *TFE3*-fusion RCC tumors relative to clear cell RCC tumors, based on higher phosphorylated levels of the downstream mTORC1 target, S6.[88] We have similarly observed the frequent activation of mTORC1 signaling in a panel of *TFE3*-fusion RCC cell lines, with variable suppression of cancer cell growth using a selective mTORC1 inhibitor.[89] Additionally, phosphorylation and nuclear localization of TFEB was recently found by Pena-Llopis *et al* to be regulated by mTORC1 in some cell types.[77] Drugs with selective mTORC1 inhibition have been studied in a small number of *TFE*-fusion patients, with occasional but inconsistent patient responses.[90,91]

6.2 TFE and Metabolic Regulation

Kidney cancer is a metabolically driven disease, as evidenced by the involvement of many kidney cancer genes within major metabolic pathways.[92] Similarly, both TFE3 and TFEB appear to play roles in regulating metabolic pathways. (Figure 5) In liver and muscle, TFE3 governs insulin signaling and glucose metabolism through upregulation of IRS-2 and the hexokinase enzymes, inhibiting lipogenesis and increasing glycogen synthesis.[93,94] We have also shown in a Birt-Hogg-Dubé preclinical model that TFE3 activity may be inhibited by expression of Folliculin, a protein found in complexes with AMPK, the primary sensor of cell energy and a putative tumor suppressor.[95] TFEB may similarly have a key energy sensing role. The *TFEB* gene product has been identified as a master transcriptional regulator of lysosomal biogenesis and autophagy, the cellular process by which nutrients are recycled during periods of cellular starvation.[96,97]

6.3 TFE3 and Cell Cycle Regulation

There is some evidence to suggest that dysregulated *TFE3* expression might promote oncogenesis by preventing cell cycle arrest. (Figure 5) TFE3 protein is known to interact with the Rb target, E2F3, causing synergistic activation of E2F target genes and allowing escape from Rb-induced cell cycle arrest.[98] The ability of TFE3 to block the anti-mitogenic effects of TGFβ has been suggested to result from TGFβ signaling dependence on the downstream Rb protein product.[98] Potential upregulation of cyclin proteins by TFE3 may promote cell cycle progression.[98,99] Furthermore, at least one type of *TFE3*-fusion protein (*SFPQ-TFE3*) can interact with p53, a key cell cycle regulator.[100]

6.4 TFE and Met Tyrosine Kinase Regulation

Upregulation of the Met tyrosine kinase, an oncogene frequently amplified in papillary RCC, has been suggested to mediate *TFE3*-fusion oncogenicity.[101,102] (Figure 5) Tsuda *et al* showed direct activation of the Met promoter by various TFE3-fusion proteins *in vitro* leading to up to 3.5-fold increases in Met transcription.[102] However, among clinical renal tumors with *TFE3*-fusions, activated (i.e., phosphorylated) Met protein was frequently undetectable in their study. Furthermore, the FU-UR1 RCC cell line harboring a *ASPSCR1-TFE3* type 1 gene fusion required very high concentrations of a selective Met inhibitor (500 nM of PHA665752) for growth inhibition, ∼10 times higher than the IC_{50} for growth reported by Guo et al in cancer cells with Met upregulation (∼100 times higher than the IC_{50} for phosphorylation), but similar to the IC_{50} among cancer cells without Met upregulation.[103] We have similarly observed no significant growth inhibition at high concentrations of selective Met inhibitors in a panel of *TFE3*-fusion RCC cell lines.[89] Hence, Met inhibition may not be an effective mono-therapy for *TFE*-fusion RCC patients. Consistent with this, a recent clinical trial using the selective Met inhibitor, tivantinib, demonstrated poor progression-free survival (median 1.9 months) and no objective responses among six RCC patients with known translocations.[104]

6.5 Functional significance of the TFE3-fusion protein partner

An alternative mechanistic hypothesis argues that the function of the *TFE3*-fusion partner may have significance within the chimeric protein. This theory has been proposed by some investigators based on functional similarity among known *TFE3* fusion partners. Most of these fusion partners appear to have regulatory roles in mRNA splicing and/or mitosis. Both NONO and SFPQ are pre-mRNA splicing factors, while PRCC can also be found in complexes with splicing factors.[24,105] Both PRCC and CLTC are implicated in mitosis control through their direct or indirect interactions with MAD2B, a regulator of the anaphase-promoting complex. PRCC binds to MAD2B directly, while CLTC is thought to regulate MAD2B through shared interactions with the clathrin light chain component (CLTA).[106,107] The similarity of functions among *TFE3* fusion partners is intriguing and warrants further investigation.

7 Conclusion

Nearly two decades since the discovery of *TFE3* gene fusions and one decade since the discovery of *TFEB* gene fusions, the mechanisms underlying the oncogenic effects of these

mutations in kidneys remain largely unclear. As with other fusion proteins involving transcription factors, promoter substitution appears to be the key molecular event with *TFE*-fusions, causing dysregulated TFE protein activity. Yet how this dyregulation translates into cancer is unknown. It is interesting in this regard that so many signaling pathways already implicated in carcinogenesis appear to be regulated by *TFE3* and *TFEB*, and it may be this multitude of pathways that allows a single mutation in these genes to be sufficient enough for cellular transformation. The ability of TFE3 and TFEB to regulate metabolic pathways and mTOR signaling is particularly intriguing, given the known roles of both processes in RCC tumorigenesis. Whether dysreguation of these pathways contributes to *TFE*-fusion carcinogenesis warrants further investigation, with an ultimate goal of pinpointing the most promising molecular targets for novel therapeutics. The advent of new and rapid genetic analysis and large scale investigative projects, such as the Cancer Genome Atlas, will identify ever increasing numbers of patients with tumors harboring gene fusions such as the *TFE*-fusion RCCs and thus now could be the optimal time to begin thorough investigations of targeted therapies based on the existing and forthcoming knowledge of these specific cases.

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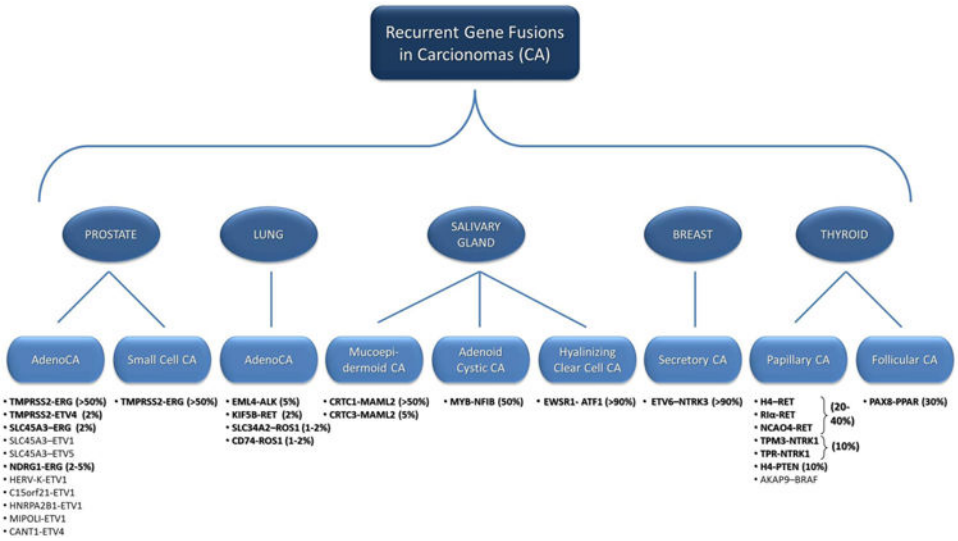

Figure 1. Recurrent Gene Fusions in Non-Renal Carcinomas

This demonstrates the currently known gene fusions that have been recurrently shown in Prostate, Lung, Salivary Gland, Breast and Thyroid carcinomas. The listed gene fusions appear in boldface if they occur with an incidence of greater that 1% with the reported percentage shown in parenthesis.

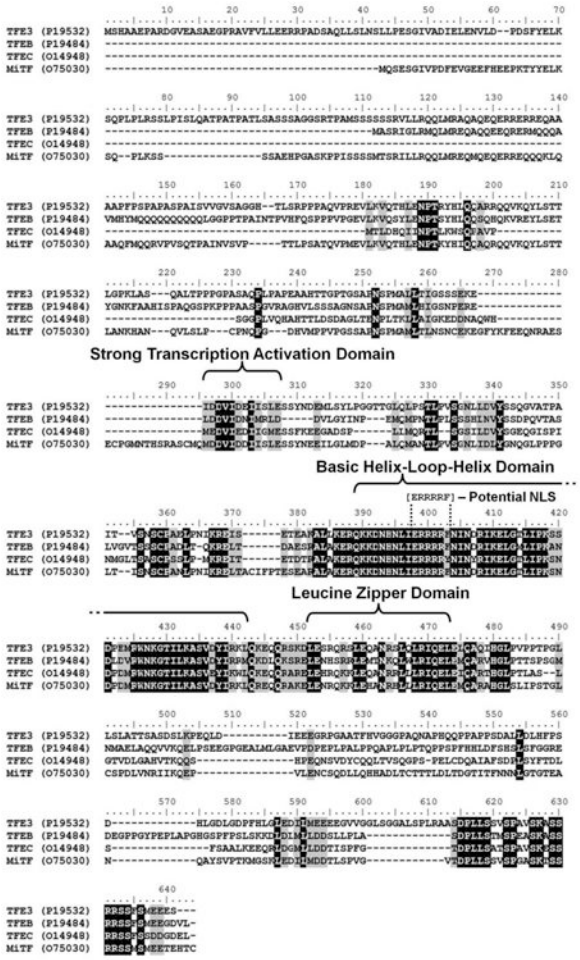

Figure 2. ClustalW Alignment of the *TFE3, TFEB, TFEC,* and *MiTF* gene protein sequences

Multiple sequence alignment using the protein sequence of the four genes of the micropthalmia transcription factor (MiT) gene family (*TFE3*, *TFEB*, *TFEC*, and *MiTF*) was performed using the ClustalW function of Bioedit Sequence Alignment Editor (http://www.mbio.ncsu.edu/bioedit/bioedit.html). The shared protein functional domains are highlighted as well as the potential nuclear localization signal (NLS) within the commonly retained region. Black shading = 100% homology, gray shading = 75% homology.

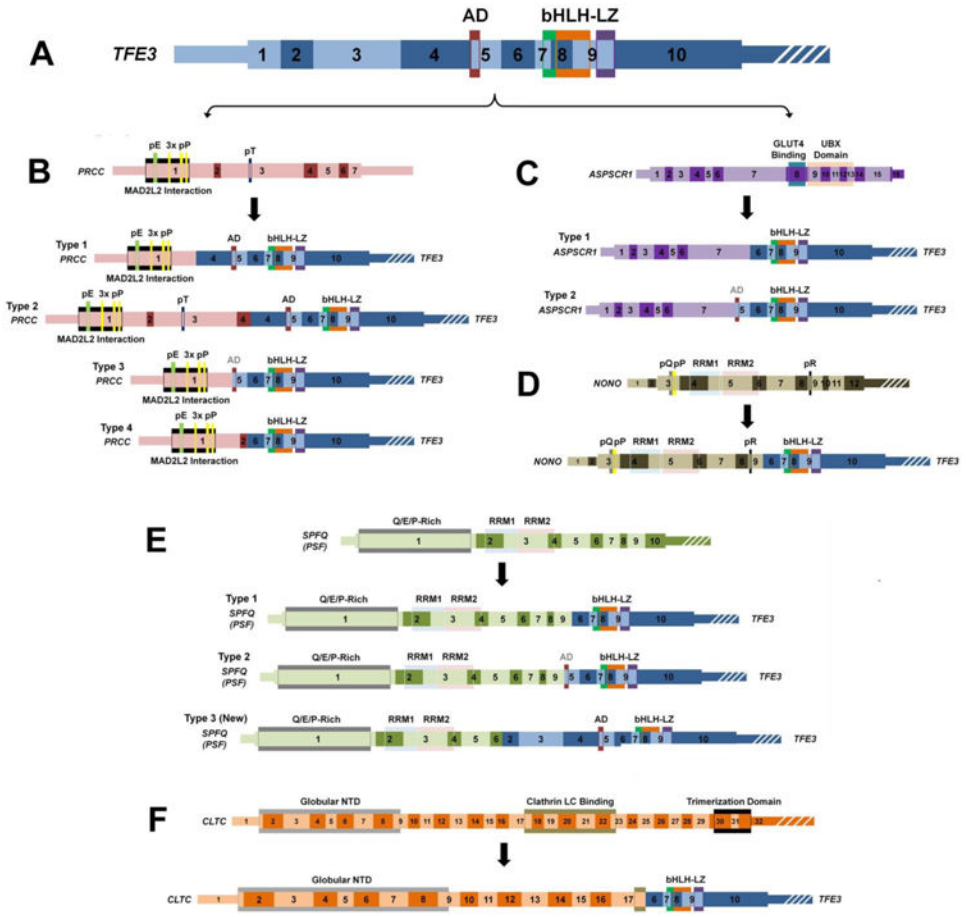

Figure 3. A schematic of the known *TFE3* gene fusions

This scale schematic demonstrates the exons and functional domains of the *TFE3* gene (A - blue), *PRCC* gene (B - pink), *ASPSCR1* gene (C - purple), *NONO* gene (D - dark green), *SPFQ* (*PSF*) gene (E - light green), *CLTC* gene (F - orange), and highlights the region/type of fusion with the *TFE3* gene. The known fusion genes are shown below each specific partner gene to demonstrate the retained exons/function domains for each fusion gene. The strong transcription activation domain (AD) crosses an exon boundary and is shaded black if all of the domain is retained and grey if only part of the domain is retained. Thin regions represent non-coding sequence, while thick regions represent the translated reading frame and white strips indicate the region is no longer to scale. bHLH = Basic Helix-Loop-Helix Domain, LZ = Leucine Zipper Domain, pE = Poly-Glutamate, pP = Poly-Proline, pT = Poly-Threonine, pQ = Poly-Glutamine, pR = Poly-Arginine, UBX = Ubiquitin Regulatory X, RMM = RNA-Recognition Motif, NTD = N-Terminal Domain.

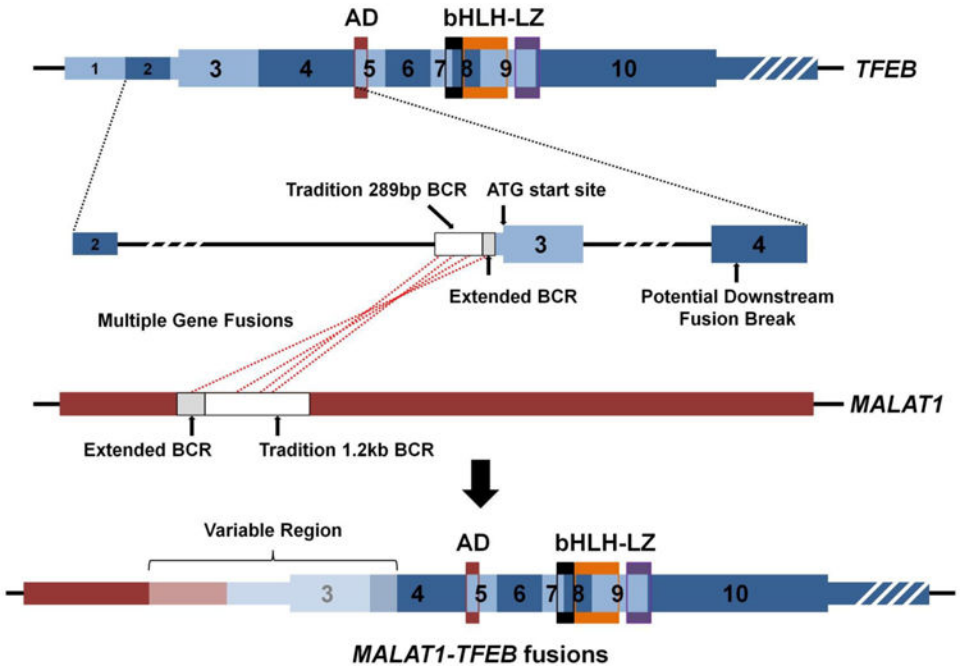

Figure 4. A schematic of *MALAT1-TFEB* gene fusions

This scale schematic demonstrates the exons and functional domains with the *TFEB* gene and highlights the regions of fusion with the non-coding *MALAT1* gene. The white box regions represent the traditional breakpoint cluster region (BCR) and the grey boxed regions represent the recently published extensions to BCR (Inamura et al[43]). To date, all but one fusion has occurred before the initial ATG translational start of *TFEB* with a single downstream fusion breakpoint reported in exon 4 (Inamura et al[43]). Thus, all *MALAT1-TFEB* gene fusions contain an upstream *MALAT1* region (red) and the majority of the *TFEB* gene (blue) with differing amounts of the "variable region" (light red/blue).

Thin regions represent non-coding sequence, while thick regions represent the translated reading frame and white strips indicate the region is no longer to scale. AD = Strong Transcription Activation Domain, bHLH = Basic Helix-Loop-Helix Domain, LZ = Leucine Zipper Domain.

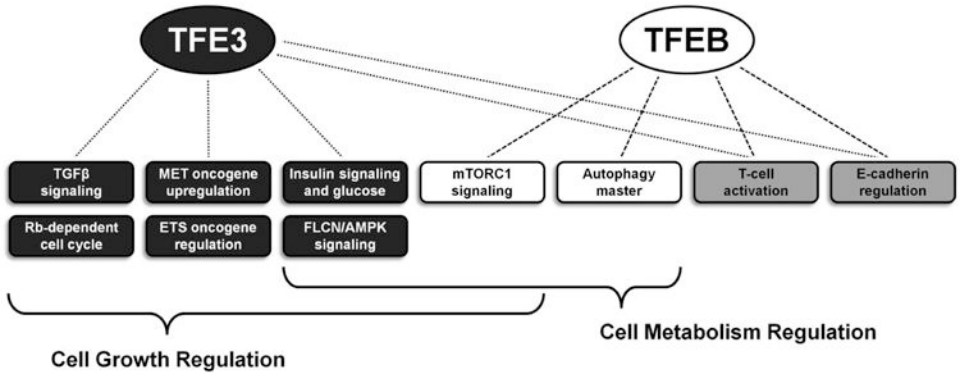

Figure 5. *TFE3* and *TFEB* functions that could potentially contribute to caricinogenesis

The functions and pathways associated solely with *TFE3* are highlighted in dark grey boxes and those solely associated with *TFEB* in white boxes. The light grey boxes represent functions and pathways associated with both genes and the brackets highlight the multiple pathways involved in more broad global pathways such as growth and metabolism.

Table 1
Mutations of MiT family of transcription factors observed clinically

MiT member	Chromosome	Mutations in RCC	Mutations in other neoplasms
MiTF	3p14.1	Germline activating amino-acid substitution	*Melanoma*: germline activating amino-acid substitution, gene amplification
TFE3	Xp11.2	Gene fusions with *PRCC, ASPSCR1, SFPQ, NONO, CLTC*	*Alveolar soft part sarcoma*: gene fusions with *ASPSCR1* *Perivascular epithelial benign tumors* (a.k.a., PEComas): gene fusions with SFPQ/other genes?
TFEB	6p21.2	Gene fusion with *MALAT1*	None known
TFEC	7q31.2	None known	None known

Table 2

TFE3 Gene-Fusions in RCC

Chromosomal Rearrangement	Gene Fusion	Type	Cell Line	Fusion Gene		Fusion Protein		Reference
				5′ Gene Exons	TFE3 Exons	Animo Acids	Predicted Mass	
t(X;1)(p11.2;q21)	*PRCC-TFE3*	1	UOK120, UOK146	1	4-10	553	58.4 kDa	Sidhar *et al*[21], Argani *et al*[36]
	PRCC-TFE3	2	UOK124	1-4	4-10	790	85.6 kDa	Sidhar *et al*[21], Argani *et al*[26]
	PRCC-TFE3	3	-	1	5-10	471	49.8 kDa	Argani *et al*[36]
	PRCC-TFE3	4	-	1-2	6-10	452	47.8 kDa	Argani *et al*[36]
t(X;1)(p11.2;p34)	*SFPQ-TFE3*	1	UOK145	1-9	6-10	942	102.0 kDa	Clark *et al*[24]
	SFPQ-TFE3	2	-	1-9	5-10	977	105.7 kDa	Chang *et al*[37], Zhong *et al*[38]
	SFPQ-TFE3	3	-	1-6	2-10	1,102	118.0 kDa	Cancer Genome Atlas Research Network[11]
t(X;17)(p11.2;q25)	*ASPSCR1-TFE3*	1	FU-UR1	1-7	6-10	591	63.7 kDa	Ladanyi *et al*[27]
	ASPSCR1-TFE3	2	-	1-7	5-10	626	67.4 kDa	Ladanyi *et al*[27]
inv(X)(p11.2;q12)	*NONO-TFE3*	-	UOK109	1-9	6-10	657	75.1 kDa	Clark *et al*[24]
t(X;17)(p11.2;q23)	*CLTC-TFE3*	-	-	1-17	6-10	1,212	136.1 kDa	Argani *et al*[36]