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Perspective

The MITF family of transcription factors: Role in endolysosomal biogenesis, Wnt signaling, and oncogenesis

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ABSTRACT

Canonical Wnt signaling influences cellular fate and proliferation through inhibition of Glycogen Synthase Kinase (GSK3) and the subsequent stabilization of its many substrates, most notably β-Catenin, a transcriptional co-activator. MITF, a melanoma oncogene member of the microphthalmia family of transcription factors (MiT), was recently found to contain novel GSK3 phosphorylation sites and to be stabilized by Wnt. Other MiT members, TFEB and TFE3, are known to play important roles in cellular clearance pathways by transcriptionally regulating the biogenesis of lysosomes and autophagosomes via activation of CLEAR elements in gene promoters of target genes. Recent studies suggest that MITF can also upregulate many lysosomal genes. MiT family members are dysregulated in cancer and are considered oncogenes, but the underlying oncogenic mechanisms remain unclear. Here we review the role of MiT members, including MITF, in lysosomal biogenesis, and how cancers overexpressing MITF, TFEB or TFE3 could rewire the lysosomal pathway, inhibit cellular senescence, and activate Wnt signaling by increasing sequestration of negative regulators of Wnt signaling in multivesicular bodies (MVBs). Microarray studies suggest that MITF expression inhibits macroautophagy. In melanoma the MITF-driven increase in MVBs generates a positive feedback loop between MITF, Wnt, and MVBs.

1. Introduction – Wnt signaling

The Wnt signaling pathway plays fundamental roles in cell biology. During development, Wnt regulates stem cell differentiation, embryonic patterning and organogenesis [1–3]. Wnt also regulates adult tissue homeostasis, and dysregulation of this pathway often leads to diseases such as cancer, in which Wnt signaling is frequently hyperactivated [1,4]. In the absence of Wnt signaling, β-Catenin is phosphorylated by Casein Kinase 1α (CK1α) and Glycogen Synthase Kinase 3 (GSK3) in a "destruction complex" which also contains the scaffold proteins Adenomatous Polyposis Coli (APC) and Axin1 [1]. These succesive phosphorylations are recognized as a phosphodegron by Beta-Transducin Repeat Containing E3 Ubiquitin Protein Ligase (β-TRCP), which ubiquitinates β-Catenin and targets it for proteasomal degradation. In this way, without Wnt stimulation, the destruction complex maintains cytosolic β-Catenin levels in check. When cells are subject to Wnt stimulation, Wnt ligands bind to their receptors low-density lipoprotein receptor-related 5 or 6 (LRP5/6) and Frizzled (Fz), resulting in the recruitment of the destruction complex, clustering, and subsequent endocytosis of activated receptor complexes into "Wnt-signalosomes" [5].

This complex is then trafficked inside multivesicular bodies (MVBs) in an Endosomal Sorting Required for Transport (ESCRT)-dependent manner [6–8]. The sequestration of GSK3 and Axin inside MVBs is required for Wnt signaling, highlighting the importance of late endosomes as positive regulators of growth factor signaling [6,9]. In this way, the destruction complex is sequestered from the cytosol and newly synthesized β-Catenin is allowed to accumulate and translocate to the nucleus, where it acts as a transcriptional co-activator that regulates the transcription of Wnt-target genes.

2. A plethora of GSK3 phosphorylations

Wnt signaling promotes stabilization of many proteins in addition to β-Catenin [6,10,11]. A bioinformatic screen of the human proteome revealed that up to 20% of human proteins contain three or more putative consecutive GSK3 phosphorylation sites (much more than predicted by chance alone), which have the

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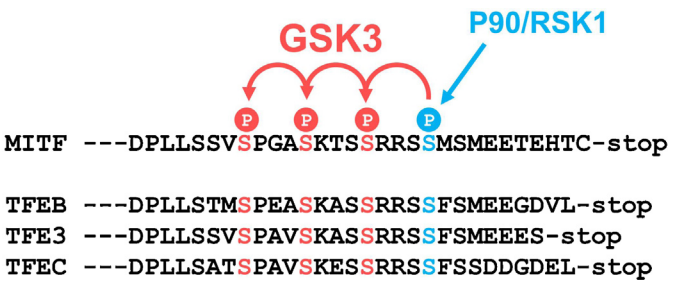

Fig. 1. MITF contains three novel GSK3 phosphorylation sites in its C-terminal region (in red), which can be primed by p90/RSK1 phosphorylation (in blue). The p90/RSK enzyme is also known as MAPK-Activated Protein Kinase 1, or MAPKAP-K1, and is a downstream target of the Ras/Erk signaling pathway. The GSK3 sites, as well as the priming phosphorylation site are present in the other members of the MiT family (TFEB, TFE3, TFEC). These GSK3 sites have the highest degree of conservation for GSK3 sites in phylogenetic proteome analyses, and are even found in insects [8].

potential of bringing the degradation of many cellular proteins under the control of Wnt (http://www.hhmi.ucla.edu/derobertis/EDR_MS/GSK3%20Proteome/Table_1-full_table.xls) [6]. As cells need to conserve their proteins in preparation for cell division, Wnt signaling is particularly strong at the G2/M of the cell cycle [12]. Furthermore, Wnt signaling can promote cell size growth independently of β-Catenin in cancer cells with constitutively stabilized β-Catenin. This new branch of canonical Wnt signaling, independent of β-Catenin-driven transcriptional effects, has been recently designated the Wnt-dependent STabilization Of Proteins (Wnt/STOP) pathway [11].

Analysis of the putative GSK3-regulated phosphoproteome revealed that the microphthalmia-related (MiT) family of basic helix-loop-helix leucine zipper (bHLH-LZ) transcription factors possessed the most phylogenetically conserved GSK3 phosphorylation sites [8]. As shown in Fig. 1, three consecutive phosphorylation sites are present in the highly conserved carboxy-terminus of the MiT member MIcrophthalmia-associated Transcription Factor (MITF) as well as in its paralogues TFEB, TFE3, and TFEC. MITF is the master regulator of melanocytes, and its levels act as a rheostat to control cellular proliferation in melanomas [13–15]. Using a custom-made phospho-specific antibody, these residues were shown to be phosphorylated by GSK3 and to mediate Wnt-induced protein stability, underscoring the predictive capacity of the putative GSK3 phosphoproteome, and reinforcing the concept of the Wnt/STOP pathway [8].

3. The MiT family of transcription factors

The Microphthalmia family of bHLH-LZ transcription factors (MiT) is composed of four members: MITF, TFEB, TFE3 and TFEC [13,16]. These proteins can form homo- or hetero-dimers through their HLH-LZ motif with members within the family, but not with other related bHLH proteins such as c-Myc, Max or USF [17,18]. Their basic domain enables them to bind to DNA, specifically E-boxes (Ephrussi boxes) and regulate the transcription of target genes involved in a wide range of different cellular processes, from pluripotency [19] and cell specific differentiation [13] to basic cellular homeostasis [20]. Importantly, MiT family members are frequently dysregulated in many forms of cancer [21].

3.1. MITF

MITF is an evolutionarily conserved transcription factor with homologs in *Caenorhabditis elegans* and *Drosophila* [22]. Paula Hertwig described the first MITF mutation 70 years ago, when she found that progeny of X-ray irradiated mice had white coat color and small eyes [23]. Since then, much has been discovered about MITF, resulting in a vast body of literature [13,16].

The *mitf* gene is predominantly expressed in melanocytes and retinal pigment epithelial cells (RPE), but is also expressed in a variety of other cell types (osteoclasts, natural killer cells, macrophages, mast cells, B cells, and cardiac muscle cells) [16]. MITF is subject to differential splicing and differential promoter usage, giving rise to multiple isoforms that differ in their first exon. Many of these isoforms show a tissue-restricted expression pattern. For example, MITF-M is an isoform preferentially expressed in melanoblasts and melanocytes, MITF-D is mainly expressed in the RPE and monocyte lineage [24], and MITF-mc is expressed in mast cells [25], while MITF-A and MITF-H are more ubiquitous, being expressed in several tissues [13,16].

Most of the current knowledge on MITF comes from studies in melanocytes and melanomas, in which the MITF-M isoform functions as a master gene regulator of the melanocytic lineage required for the development, growth and survival of melanocytes [13,16]. Mutations in the *mitf* gene can give rise to mice that have a white coat color, are deaf, and present microphthalmia [13,16]. In humans, heterozygous mutations in *mitf* can cause Waardenburg Syndrome type 2, a hair pigmentation and deafness disorder [26], and dominant-negative mutations cause Tietz syndrome, a closely related condition characterized by generalized depigmentation and deafness [27]. Importantly, genomic amplification of *MITF* is observed in 15–20% of melanomas, in which it functions as a lineage addiction oncogene [28].

3.2. TFEB

Transcription Factor E Box (TFEB), the second best characterized member of the MiT family, plays a crucial role in cellular homeostasis and has been recently described as the master regulator of cellular clearance pathways [29,30]. TFEB achieves this by transcriptionally upregulating genes involved in lysosome and autophagosome biogenesis, as well as by activating lysosomal exocytosis and lipid metabolism [31–34]. TFEB provides a link between the nutrient-sensing mechanistic Target of Rapamycin Complex 1 (mTORC1) machinery and the transcriptional cellular response needed to cope with nutritional stress. In nutrient-rich conditions, active mTORC on the outer lysosomal membrane phosphorylates TFEB, inhibiting its nuclear translocation. Upon starvation, mTORC is inhibited and unphosphorylated TFEB accumulates in the nucleus, where it binds to Coordinated Lysosomal Expression And Regulation (CLEAR) sequences located in the promoters of lysosomal genes and activates the expression of the suite of genes necessary for cells to survive nutrient deprivation [30,35].

3.3. TFE3

Like TFEB, Transcription Factor E3 (TFE3) has been shown to participate in the biogenesis of lysosomes and autophagosomes and the clearance of cellular debris under conditions of starvation or lysosomal stress [36]. The activity of TFE3 is also dictated by its nuclear localization, which is regulated by mTOR and nutrient levels. When active, mTOR phosphorylates TFE3 causing its cytoplasmic retention. In conditions of nutritional deprivation, mTORC is inhibited and unphosphorylated TFE3 can enter the nucleus and activate the transcription of genes containing CLEAR elements in their promoters [37]. TFE3 has also been recently described to play a role in the maintenance of pluripotency [19]. A relocalization of TFE3 from the nucleus to the cytoplasm was found to be required for embryonic stem cells (ESC) to exit their naïve pluripotent state and commit to cellular differentiation. The cytoplasmic retention of TFE3 is controlled by the activity of the tumor suppressor

Folliculin, its binding partners Folliculin-interacting proteins 1 and 2 (Fnip1/2), and the mTORC regulator TSC2 [19,38].

3.4. TFEC

Of the four members of the MiT family, TFEC is the least studied one. Its expression is restricted to macrophages [39], and its function has not been widely investigated. Furthermore, while mouse and rat TFEC are devoid of the activation domain required for promoting transcription [39–41], the human homologue does contain this activation domain and is able to activate expression of a reporter gene construct driven by the *tyrosinase* or *heme oxygenase-1* gene promoters [42].

4. MiT transcription factors drive lysosome and autophagosome biogenesis

As mentioned above, TFEB and TFE3 have been clearly established as regulators of lysosomal biogenesis. TFEB and TFE3 are recruited to the outer lysosomal membrane by interacting, through their 30-most N-terminal residues, to Rag guanosine triphosphatases (GTPases) [43]. In nutrient-rich conditions, active mTORC1 is recruited to the surface of lysosomes [44], where it phosphorylates Ser142 in TFEB (and also possibly Ser211 in TFEB and Ser321 in TFE3), triggering the binding of these transcription factors to 14-3-3 cytosolic chaperones [45]. This causes cytoplasmic retention of TFEB and TFE3, resulting in decreased transcriptional activity [30,36]. However, when cells are subject to nutritional challenges (such as amino acid starvation) or lysosomal stress (such as chloroquine treatment), mTORC activity is inhibited, and unphosphorylated TFEB and TFE3 translocate to the nucleus, bind to CLEAR elements, and drive the coordinated transcription of gene programs that restore nutritional homeostasis through an increase in lysosomes, autophagosomes, and exocytosis [30,36].

5. MITF expression correlates with many endolysosomal genes

In recent work, MITF has also been implicated in lysosomal biogenesis [8]. Using Gene Set Enrichment Analysis (GSEA) of microarray expression datasets from 51 different melanoma lines analyzed at UCLA [8], MITF levels were found to correlate with the expression of many, but not all lysosomal genes containing CLEAR elements in their promoters/enhancers (Fig. 2). Intriguingly, TFEB levels did not correlate with lysosomal gene expression in melanoma microarray datasets ($p = 0.276$) [8]. Furthermore, overexpression of MITF-M was able to drive transcription of lysosomal markers and activate a CLEAR element reporter in melanoma cells. Surprisingly, despite a robust increase in endolysosomes/MVBs induced by MITF-M, these organelles are not completely functional lysosomes because they failed to degrade endocytosed proteins [8].

There were other indications suggesting a role for MITF isoforms in regulating CLEAR element lysosomal genes. MITF-A has been shown to localize at the lysosomal membrane, bind to Rag GTPases, and translocate to the nucleus upon mTOR inhibition, mimicking the nutrient-driven regulation of TFEB and TFE3 [43]. In the case of TFEB, Ser142 and Ser211 were known to be required for cytoplasmic retention, as they are the sites for mTORC phosphorylations which promote association with 14-3-3 proteins [45,46]. Interestingly, Ser173 in MITF, which is analogous to Ser211 in TFEB, has also been shown to be required for cytoplasmic retention by interacting with 14-3-3 proteins in myeloid precursors [47]. It is important to note that while the activity of TFEB, TFE3, and MITF isoforms A and D is regulated by nutritional status which determines cytoplasmic versus nuclear localization through mTOR phosphorylations, the MITF-M isoform, which is the one enriched in melanomas, escapes this regulation as it lacks the amino-terminal domain required for binding to Rag GTPase and lysosomal docking [43,46]. Thus, MITF-M is a constitutively nuclear transcription factor. MITF-A overexpressed in the human retinal pigment epithelium cell line ARPE-19 effectively activates the expression of many autophagy genes in this cell type [37].

6. MITF expression negatively correlates with key macroautophagy genes

Autophagosomal genes did not significantly correlate with MITF expression. In Supplementary Table 1, we list the rank order of 13,214 genes arranged according to their degree of correlation with MITF across 83 different melanoma cell lines in microarrays. Examination of 39 autophagosomal genes [32] showed that while some were positively correlated (ATG3, UVRAG, VPS11, SNCA, and AMBRA1) many others (e.g., ATG7, ATG12, ATG5) were very negatively correlated with MITF. In GSEA analyses [48] the set of autophagy genes had no significant correlation ($p = 0.314$) with MITF, while lysosomal genes were highly correlated ($p < 0.001$) [8].

Supplementary table related to this article can be found, in the online version, at http://dx.doi.org/10.1016/j.phrs.2015.04.006

The observation that the MITF in melanomas (MITF-M) correlates with lysosomal genes but not with autophagy genes is surprising, given the fact that the CLEAR element (-CTCACGTGAG-) contains one of the two DNA binding sequences for MITF (-CACGTG-), as defined from ChIP-seq experiments from melanoma cells [15,31]. In addition, the CLEAR element also contains a 5′ flanking T known to be crucial for MITF binding [49]. A further layer of mystery is added by the fact that in RPE cells MITF-A, which can be regulated by nutrient starvation, activates autophagy but not lysosomal genes [37].

ChIP-seq experiments in melanoma have uncovered potential roles for MITF in regulating DNA replication, mitosis, and genomic stability. In these datasets MITF was found bound to promoters of many lysosomal genes [15]. Furthermore, DNA microarray analysis of MITF targets had also identified lysosomal genes such as acid Ceramidase (ASAH1), GM2 ganglioside activator (GM2A), and various ATPases [50]. Finally, MITF was known to activate transcription of the lysosomal genes acid phosphatase 5 (ACP5), chloride channel voltage-sensitive 7 (CLCN7), osteopetrosis-associated transmembrane protein 1 (OSTM1) and cathepsin K [51–53]. Although MITF is known to regulate the expression of many genes required for melanosome biogenesis, and melanosomes are frequently referred to as a lysosome-related organelle (LRO), melanosomes are quite different from conventional endosomes and lysosomes, and represent a distinct lineage of organelles with a unique proteomic profile [54–57]. Thus, the transcriptional regulation of the lysosomal gene set by MITF is not merely an upregulation of melanosomal genes.

Since MiT members can bind to similar core DNA sequences and hetero-dimerize with each other, there are possibilities for crosstalk and compensation among members. For example, MITF and TFE3 have been shown to be redundant in osteoclasts [58]. In conclusion, the MiT family members TFEB, TFE3 and MITF regulate lysosomal and autophagy genes, but the contribution of each member, or each isoform in the case of MITF, to organelle biogenesis may depend on nutritional conditions, cell type and signaling inputs.

7. The MiT family in cancer

MITF, TFEB and TFE3 are oncogenes. MITF is amplified in 20% of melanomas, while TFEB and TFE3 are driven by translocations in pediatric renal cell carcinomas and alveolar soft part sarcomas [21].

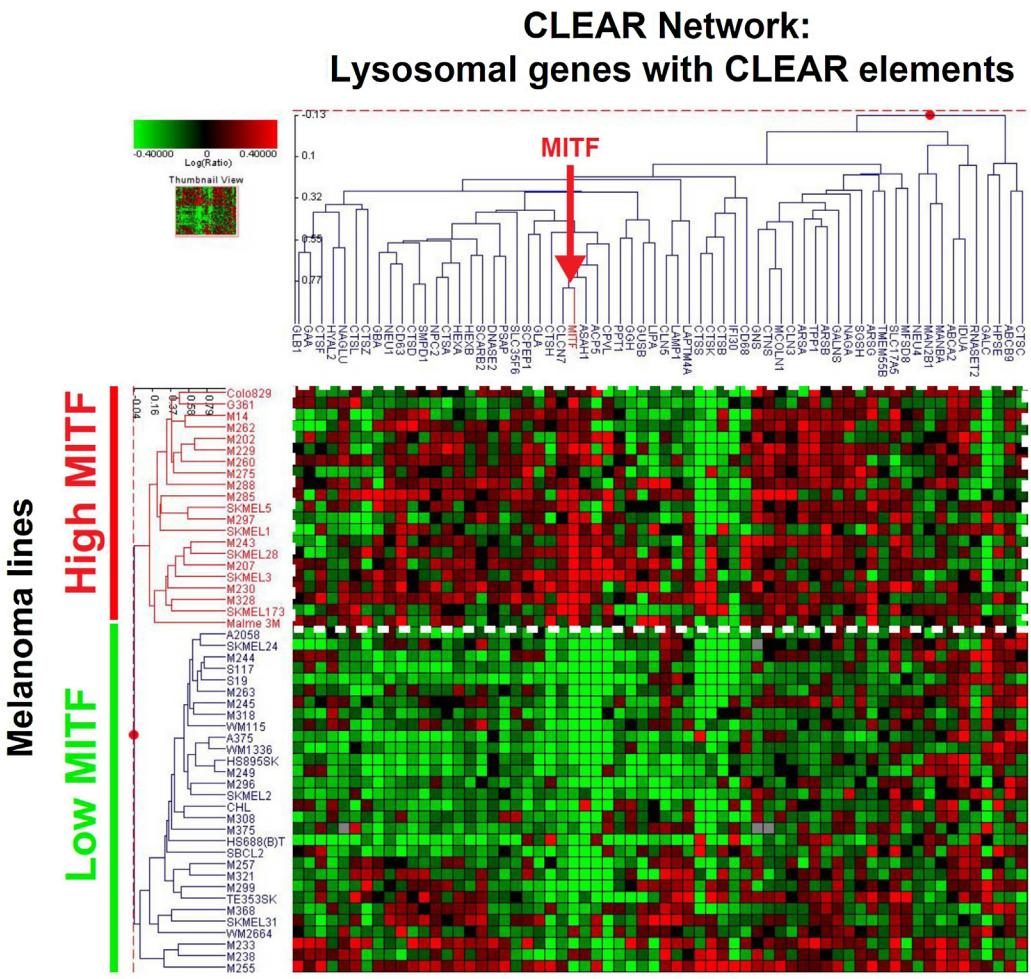

Fig. 2. In melanoma cell lines *MITF* expression correlates with the expression of many lysosomal genes containing a CLEAR promoter element. This microarray analysis shows that 51 melanoma cell lines clustered into two distinct groups according to their expression signature of 63 CLEAR element lysosomal genes (Sardiello et al., 2009 [31]). One group had high *MITF* expression (in red) and the other had low *MITF* expression (in green). The melanoma group with high *MITF* expression levels upregulated many, but not all, lysosomal genes containing a CLEAR element in their promoter. Horizontal lines in this heat map correspond to individual cell lines, and vertical lines show lysosomal gene expression levels. *MITF* expression was added as a reference, for it is not a lysosomal gene. The cell lines were allowed to sort according to their lysosomal gene expression signature. Surprisingly, all cell lines with high *MITF* mRNA expression (mostly through *MITF* amplification) clustered as a high lysosomal gene expressing group (above the dotted white line).

Reproduced from the Supplementary information of Ploper et al. (2015) [8].

In pediatric renal carcinomas, TFE3 is translocated in up to 30–50% of cases [59,60].

Through systematic analysis of cancer genomes, melanomas were found to have amplifications of chromosome 3p14-3p12, which coincides with the MITF locus [28]. MITF is amplified in 20% of metastatic melanomas, one of the most aggressive and chemo resistant cancers known, and associated with decreased patient survival. Furthermore, overexpression of MITF is able to cooperate with $BRAF^{V600E}$ activating mutations in transforming human melanocytes in vitro. This places MITF in the category of a "lineage survival", or "lineage addiction" oncogene, since in addition to its oncogenic effects it is required for the development and survival of the melanocytic lineage [28,61].

A point mutation in *MITF*, E318K, predisposes to familial and sporadic melanoma [62]. This mutant MITF protein cannot be properly SUMOylated and has increased transcriptional activity [62,63]. The ability of MITF to promote oncogenesis is not restricted to the melanocyte lineage, since the $MITF^{E318K}$ mutant also predisposes to renal cell carcinoma [63]. Furthermore, MITF is ectopically overexpressed in clear cell sarcomas when its transcription is activated by a fusion protein resulting from chromosomal translocation of the Ewing Sarcoma protein (EWS) to Activating Transcription Factor 1 (ATF1) [64]. Although these sarcomas do not have MITF genomic rearrangements, they are entirely dependent on MITF transcription and activity for proliferation [65].

In melanomas, MITF is known to act as a molecular rheostat; at very low levels it will causes cellular senescence, while at very high levels it can drive differentiation and growth arrest. At intermediate/high levels, however, MITF promotes cellular growth [13–15]. MITF has been proposed to promote oncogenesis by influencing multiple cellular processes, regulating the expression of genes involved in proliferation, survival, motility, oxidative stress and DNA repair, all of which are key processes in carcinogenesis [21].

MITF-M overexpression in melanoma cells causes an expansion of the late endolysosome/MVB compartment which enhances Wnt signaling by increasing the sequestration of destruction complex components, including Axin1, GSK3, and phospho-β-Catenin inside MVBs [8]. As depicted in Fig. 3, in the presence of Wnt a positive feedback loop is generated, in which MITF stabilized by Wnt increases MVB biosynthesis which in turn potentiates Wnt signaling [8].

A recent study found that melanomas rely on lysosomal function more than other cancers [66]. Interestingly, the related MiT transcription factors TFEB and TFE3, master lysosomal regulators, can

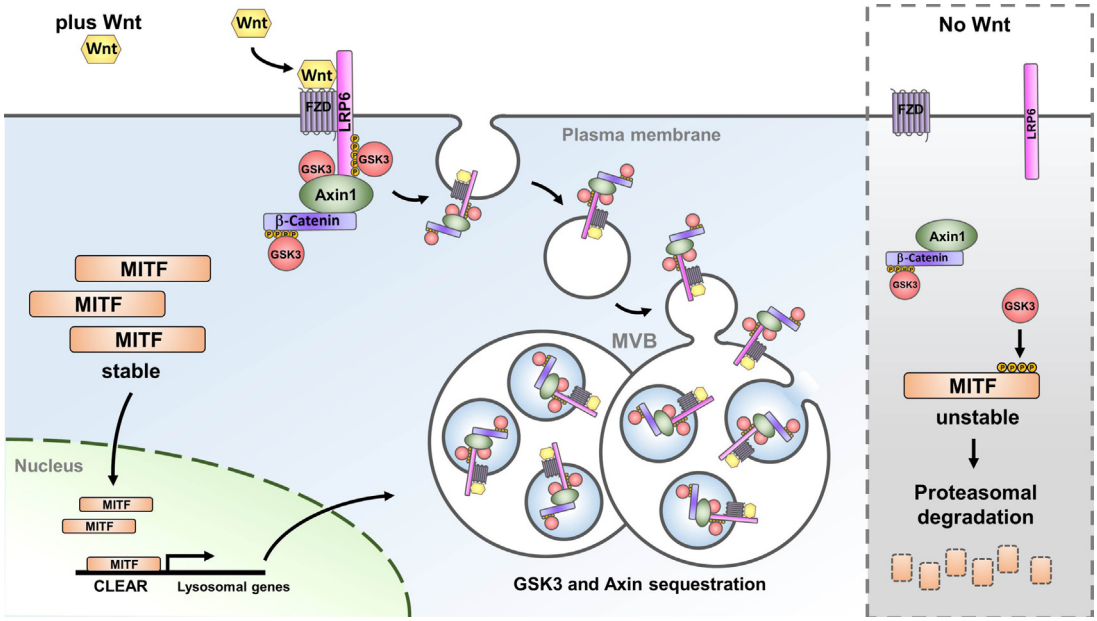

Fig. 3. Diagram showing how MVB/endolysosomal membrane trafficking regulates MITF and other Wnt/STOP targets through the sequestration of GSK3 and Axin. Upon Wnt ligand binding, the destruction complex that phosphorylates and targets β-Catenin for proteasomal degradation is recruited to the LRP6 receptor complex, which is endocytosed and subsequently sequestered inside intraluminal vesicles of multivesicular bodies (MVBs). The sequestration of GSK3 and Axin1 stabilizes MITF protein, which can constitutively translocate into the nucleus and promote expression of a large subset of lysosomal genes. This expands the MVB compartment which can further sequester destruction complex components and enhance the Wnt signal. In the absence of Wnt, GSK3 phosphorylates MITF at the C-terminal sites, promoting its degradation in proteasomes.

rescue MITF knockdown in MITF-driven clear cell sarcomas [65]. Likewise, knockdown of TFE3 inhibits growth in *TFE3*-translocated renal cell carcinomas while co-transfection of ectopic MITF restores proliferation in these cells [65]. In renal cell carcinomas, translocated TFEB transcription is driven at high levels and is required for the growth of these tumors [65,67]. Recently, pancreatic cancer cell lines were shown to have nuclear localization of TFEB despite culture in a nutrient-rich medium while non-transformed pancreatic epithelial cells had cytoplasmic TFEB, suggesting a role of this lysosome-inducing transcription factor in promoting pancreatic cancer cell growth [68].

Taken together, these studies argue that MiT family members are oncogenes that can compensate for each other in oncogenesis. It is unclear whether MITF, TFEB, and TFE3 are promoting oncogenic growth by targeting a common pathway or gene network in different tumors. However, it seems likely that a reprogramming of the endolysosomal/autophagic pathway is taking place given the recent findings linking MITF and other MiT members in lysosome/autophagosome biogenesis. The nature and mechanistic detail of this rewiring, and how it affects cellular proliferation and metabolism merits further investigation.

8. Lysosomes and autophagosomes in melanomas

Cancer cells have alterations in lysosomal/autophagosome composition and abundance that may participate in oncogenesis by promoting invasive growth, metabolism and angiogenesis [69]. Among all cancers, melanomas rely on the endolysosomal pathway for growth more strongly than others, and lysosomal genes (plus lysosomal-associated genes such as Rab7) are more highly expressed in melanomas compared to other tumors [66]. Rab7, a small GTPase which has pivotal roles in lysosomal biogenesis and the degradation of lysosomal-associated vesicles [70–72], has been recently shown to be an early melanoma driver capable of rewiring the endolysosomal pathway in a lineage-specific fashion [66]. Although in this study Rab7 levels were reported to be independent of MITF, MITF significantly correlates with Rab7 in microarray data presented in Supplementary Table 1 (in which it appears at position 226/13,214; $p < 0.001$), as well as with many lysosomal genes [8]. It appears that Rab7- or MITF-driven reprogramming of the endolysosomal pathway may confer growth advantages to melanomas. It is likely that other tumors that overexpress TFEB and TFE3 due to chromosomal translocations may also rewire the endolysosomal pathway.

Macroautophagy is a vital cellular process by which cells digest their own contents by engulfing cellular materials in double-membrane vesicles, called autophagosomes, which then fuse to lysosomes [73]. The role of macroautophagy in cancer is complex [74,75]. Although it is generally considered a tumor-suppressing mechanism, it can also replenish nutrients required by rapidly growing tumor cells enabling further growth [76,77]. Autophagy is regulated by highly conserved autophagy-related proteins (ATGs) required for initiation of autophagosome formation, cargo collection and trafficking to the lysosomal compartment [78]. Autophagy-Related Protein 5 (ATG5), an E3 ubiquitin ligase required for autophagosome elongation, has recently been shown to be downregulated in melanomas compared to nevi and melanocytes [79]. This is accompanied by a general inhibition of autophagy. When ATG5 is ectopically expressed in melanoma cells, it is capable of inhibiting proliferation and inducing senescence, as well as increasing autophagy [79]. In this way, autophagy in general and ATG5 in particular function as tumor suppressors in melanoma [79]. Interestingly, high MITF expression levels correlate with very low ATG5 and ATG7 levels in microarray expression data from a panel of 83 melanoma cell lines (appearing at positions 11,730 and 12,676, respectively, in Supplementary Table 1, $p < 0.001$). Macroautophagy contributes to the induction of oncogene-induced cell senescence, and melanomas with impaired autophagy and reduced ATG5 levels may escape this homeostatic mechanism [79–81]. High levels of MITF, which correlate with low levels of ATG5, might contribute to proliferation by inhibiting oncogene-induced senescence [82,83]. In conclusion, MITF

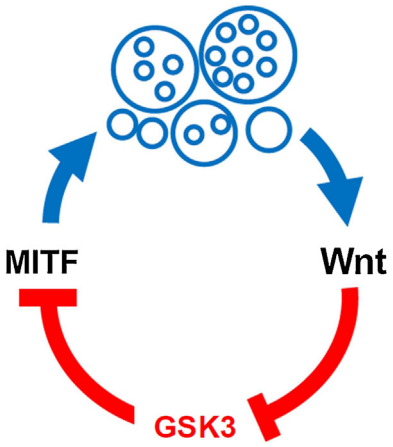

Fig. 4. Simplified model of the positive feed-back loop through which MITF increases Wnt responsiveness by the expansion of the MVB compartment. Expansion of the MVB compartment causes increased inhibition of GSK3 by enhancing its sequestration inside the late endosomal compartment. This in turn inhibits the C-terminal phosphorylations of MITF, increasing its stability and activity.

expression drives lysosomal biogenesis but may represses key macroautophagy genes in melanoma (Supplementary Table 1).

MITF, although capable of inducing late endosome/MVB formation, does not induce proteolytically active lysosomes [8]. The MITF-induced increase in MVBs in turn enhances Wnt signaling by increasing the sequestration of destruction complex components such as GSK3 and Axin, which is relevant in melanoma [8,84], resulting in increased MITF stabilization (Fig. 4). It is possible that cancers in which TFEB and TFE3 are overexpressed also expand the late endolysosomal compartment causing an increase in Wnt signaling by a similar mechanism. Chloroquine (CQ), a lysosomotropic weak base known to inhibit lysosomal acidification and function [85] also enhances Wnt signaling by increasing GSK3 sequestration [86].

9. Pharmacological approaches

Lysosomal and autophagosomal function is critical for the maintenance of proteostasis and for the ability of cells to respond to stress [77,80,81,87]. Although autophagy has been described as a tumor suppressing mechanism (given that loss of *ATG* genes promote cancer, including lymphomas and lung and liver carcinomas [88–90]), CQ and hydroxychloroquine (HCQ) (FDA-approved drugs for the prophylactic treatment of malaria, lupus erythematosus and rheumatoid arthritis) have never been linked to malignant transformation. Autophagy is also crucial for supporting progression and dissemination of established tumors, allowing cells to survive in low-nutrient and hypoxic conditions [91]. Therefore, the lysosomal/autophagosomal pathway has long been considered an attractive target for anticancer treatments. Inhibitors of lysosome/autophagosome function such as CQ, HCQ, Lys0569, Monensin, Bafilomycin A1, or of PI3K (Wortmannin) have been proposed to have possible antineoplastic effects, particularly in combination with conventional chemotherapy treatments [87,92].

Although most melanomas are initiated by activating $BRAF^{V600E}$ mutations (also present in benign nevi), MITF expression is a key driver of melanoma proliferation [93]. At intermediate levels in the MITF rheostat, melanoma cells actively proliferate, have elevated Wnt signaling, and tend not to metastasize [92]. However, at low MITF levels melanoma cell phenotypes switch to slow-proliferating stem cell-like cells with metastasizing properties. Melanomas progress through cell switching between these two cellular phenotypes that coexist in tumors in vivo [94]. Recently, MITF-driven phenotype switching has been proposed as of possible therapeutic value [95]. Induction of MITF causes expression of high levels of the enzyme Tyrosinase, the rate limiting enzyme in the synthesis of melanin. An irreversible inhibitor of dihydrofolate reductase (DHFR) has been engineered as a drug precursor (called TMECG) that is active only after being processed specifically by tyrosinase [95]. Post-translational Wnt/STOP signaling, through GSK3 inhibition, stabilizes MITF [8], and MITF is also transcriptionally upregulated by β-Catenin [96]. This suggests the possibility that it might be possible to combine the use of GSK3 inhibitors (which increase MITF activity and consequently the expression of its target gene tyrosinase), with the DHFR inhibitor TMECG in order to boost the sensitivity of the chemo-resistant invasive stem-like cells to treatment.

In addition, GSK3 inhibitors might enhance the effects of CQ on tumors highly dependent on endolysosomal activity, such as is the case in melanomas [66]. Inhibiting GSK3 activity with small molecules may be considered a double-edged sword as it could stimulate malignant transformation and cancer growth by activating Wnt/β-Catenin signaling or by stabilizing oncogenes regulated by Wnt/STOP such as c-Jun and c-Myc [6,11]. However, long-term studies in mice treated with GSK3 inhibitors have not revealed oncogenic effects [97]. Additionally, the GSK3 inhibitor Lithium Chloride, which has long been used to treat patients with bipolar disorder, has never been linked to increased cancer incidence [97]. In fact, it has been observed that GSK3 inhibitors can decrease cellular proliferation of some cancers, including melanoma, prostate, and pancreatic tumors [98,99]. GSK3 inhibitors may eventually be used as an anticancer treatment [97]; the GSK3 inhibitor LY2090314 is currently being tested in clinical trials for the treatment of metastatic pancreatic cancer [NCT01632306] and acute leukemia [NCT01214603].

10. Conclusions

Recent studies have led to the emerging concept that when the MiT members MITF, TFEB or TFEC are amplified or overexpressed in melanoma, renal cell carcinomas, clear cell sarcomas, and perhaps other cancers, the trafficking of lysosomal/autophagosome organelles and their function in proteostasis should be significantly altered. In the case of MITF overexpression, vesicular trafficking reprogramming increases MVB/late endolysosomes that enhance canonical Wnt signaling by sequestering destruction complex components, compromising lysosomal/autophagy functions, and impairs oncogene-induced senescence, conferring a growth advantage to tumor cells. Wnt-driven stabilization of proteins (Wnt/STOP) should be potentiated in these tumors, with many cellular proteins (in addition to β-Catenin) becoming more stable. In melanomas, MITF itself is stabilized, giving rise to a positive feedback regulation (Fig. 4) that further affects endolysosomal trafficking and Wnt signaling during the proliferative stages of melanoma growth [8]. One possibility is that this regulatory loop might be manipulated via GSK3 inhibitors to entice differentiation (through phenotype-switching caused by increased MITF levels) [93–95] of the slowly dividing stem-like cells that make melanomas so refractory to treatment.

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