Oncogene-induced autophagy enhances migration and invasion of breast and prostate cancer cells

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The cyclin D binding Myb like Transcription Factor 1 (*DMTF1)* is a haploinsufficient tumor suppressor present in various malignancies. DMTF1 is a known positive regulator of the $p14^{ARF}$- p53 pathway. We reported that alternative splicing generates a dominant negative, truncated version of full-length DMTF1α, named DMTF1β, with p53 dependent and independent functions. An oncogenic potential of DMTF1β was found in mammary oncogenesis.

To further study DMTF1β oncogenic functions we analyzed its protein expression levels in a breast cancer cell line panel. We found a positive correlation between DMTF1β protein expression and percentage of $CD24^{low}$ $CD44^{high}$ tumor-initiating cells (TIC) that are associated with a more aggressive phenotype. Specifically knocking down DMTF1β in $CD24^{low}$ $CD44^{high}$ aggressive MDA-MB-231 breast cancer cells reduced both the percentage of TICs and colony formation as assessed by CD24/CD44 flow cytometry, SOX2/OCT4 reporter and clonogenic assays. Moreover, reduced DMTF1β levels led to a significantly decreased migration and invasion rate in wound closure, transwell invasion and zebrafish xenograft experiments. In addition, knocking out total *DMTF1* gene expression in MCF7 breast cancer cells via CRISPR/Cas9 resulted in reduced migration and invasion, which was rescued by re-expressing DMTF1β. Of note, we reproduced similar results using PC3MPRO4 prostate cancer cells, indicating that the oncogenic role of DMTF1β is not limited to breast cancer. Together, our findings point to a DMTF1α-independent role for DMTF1β in tumor initiation, cell migration and cell invasion.

Next, we compared gene expression in PC3MPRO4 control and DMTF1β knockdown cells by RNAseq to identify molecular pathways explaining the aggressive phenotype in these cells. Interestingly, many autophagy related genes (involved at early steps of autophagy) were downregulated in DMTF1β knockdown cells, suggesting that DMTF1β might be an autophagy activator. Given that autophagy supports cellular migration and is required for stem cell quality control and homeostasis, we hypothesized that DMTF1β contributes to increased migration by activating autophagy. Indeed, autophagic flux significantly decreased in MCF7 DMTF1- null cells, but could be restored by reintroducing DMTF1β as assessed by LC3B-dependent and -independent autophagy assays. Consistent with our findings in MCF7 DMTF1-null cells, knockdown of DMTF1β in MDA-MB-231 breast and PC3MPRO4 prostate cancer cells significantly lowered autophagic flux. Furthermore, inhibiting autophagy pharmacologically or by using RNAi against key autophagy initiator kinases led to decreased migration of DMTF1β rescued cells, while untreated cells were unaffected.

In total, our findings suggest that the oncogenic DMTF1β isoform is associated with increased cellular stemness and aggressiveness. The latter notion is underscored by altered migration and invasion when DMTF1β levels are modulated. Furthermore, we show that DMTF1β-induced migration depends on autophagy activity. In conclusion, we have identified novel functions for DMTF1 isoforms in regulating cell motility and autophagy.