

Oncogenic MAGEA-TRIM28 ubiquitin ligase downregulates autophagy by ubiquitinating and degrading AMPK in cancer

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Autophagy is commonly altered in cancer and has a complicated, but important role in regulation of tumor growth. Autophagy is often tumor suppressive in the early stages of cancer development, but contributes to the late stages of tumor growth. Because of this, putative oncogenes that modulate autophagy signaling are especially interesting. Here we discuss our recent work detailing the function of the MAGEA-TRIM28 ubiquitin ligase as an oncogene product that targets PRKAA1/AMPK α 1 for ubiquitination and proteasome-mediated degradation. Degradation of AMPK, a master cellular energy sensor and regulator, by MAGEA-TRIM28 results in significantly reduced autophagy and changes in cellular metabolism, including upregulation of MTOR signaling. Overall, expression of *MAGEA3* (or *MAGEA6*) and degradation of AMPK is sufficient to induce transformation of normal cells and promote multiple hallmarks of cancer.

MAGE proteins comprise a family of approximately 50 members in humans. Our lab has previously determined that MAGE proteins function as regulators of E3 RING ubiquitin ligases, but the exact substrates and functions of individual MAGEs are largely unknown. Recently, we determined the role of the E3 ubiquitin ligase complex of MAGEA3 (or MAGEA6) with its cognate E3 ligase, TRIM28/KAP1, referred herein as MAGEA-TRIM28 ubiquitin ligase since either of the highly redundant MAGEA3 or MAGEA6 proteins can similarly function with TRIM28. We found that the *MAGEA3* and *MAGEA6* genes are normally expressed

exclusively in the testis, but are aberrantly expressed in a variety of tumor types, where their expression correlates with poor prognosis. Tumors that express *MAGEA3* or *MAGEA6* are dependent on its expression for survival, and its depletion is toxic to these cells. Through unbiased in vitro screening for substrates of the MAGEA-TRIM28 ubiquitin ligase complex, we discovered that it targets the well-known tumor suppressor AMPK for ubiquitination and degradation. Specifically, MAGEA3 and MAGEA6 bind to the PRKAA1/AMPK α 1 subunit and recruit it to the TRIM28 ligase for ubiquitination, resulting in degradation of the AMPK holoenzyme complex by the proteasome (Fig. 1, right). This degradation has dramatic alterations on many aspects of the cell, including decreased AMPK signaling, amplification of MTOR signaling, and downregulation of autophagy. In order to test the oncogenic potential of MAGEA6, we introduced it into untransformed epithelial cells and assayed for oncogenic phenotypes. To a degree similar to or even stronger than bona fide oncogene products, such as KRAS^{G12V}, *MAGEA6* was able to transform cells. Excitingly, these oncogenic activities of *MAGEA6* can be reversed by increasing AMPK activity with the addition of AMPK activating drugs such as metformin or A769662.

While our recent findings established that the MAGEA-TRIM28 ubiquitin ligase functions to inhibit autophagy, previous publications and our own findings have shown that TRIM28 functions as a pro-autophagy factor in cells not expressing *MAGEA3* or *MAGEA6* through its independent SUMO ligase activity. In

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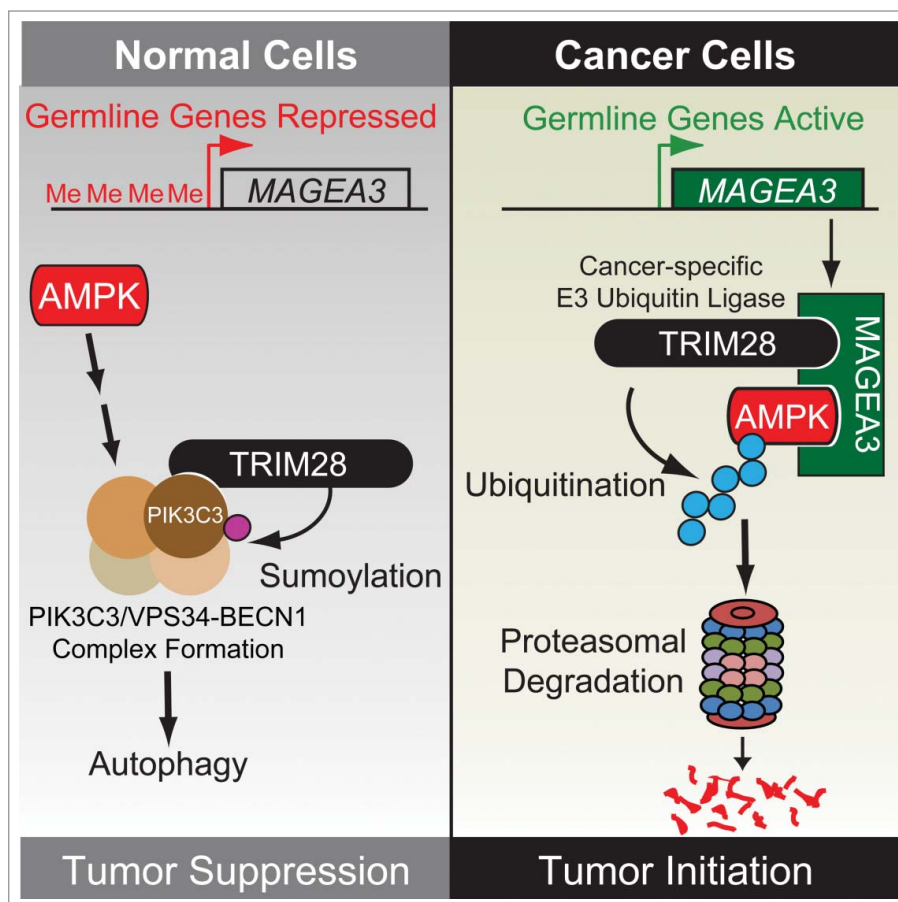


Figure 1. Model for MAGEA3 (or MAGEA6) and TRIM28 function in regulation of autophagy. Left: In normal cells outside the testis, MAGEA3 (or MAGEA6) is not expressed due to CpG promoter methylation. In the absence of MAGEA3 (or MAGEA6), TRIM28 promotes autophagy and tumor suppression through sumoylation of PIK3C3 that normally is required for assembly of the PIK3C3-BECN1 complex. This complex in combination with AMPK drives autophagy. Right: In cancer cells, MAGEA3 (or MAGEA6) is hypomethylated and transcriptionally activated. MAGEA3 (or MAGEA6) bind PRKAA1/AMPK α 1 and recruit AMPK to TRIM28 for ubiquitination and subsequent proteasomal degradation. This results in downregulation of autophagy and is permissive for tumor initiation.

these cells, TRIM28 sumoylates PIK3C3/VPS34, which promotes formation of the PIK3C3-BECN1 complex and induction of autophagy (Fig. 1, left). Our findings suggest that expression of MAGEA3 or MAGEA6 in cancer cells has the ability to act as a molecular switch to convert TRIM28 from a pro-autophagy to an anti-autophagy factor by targeting AMPK for degradation by TRIM28, which TRIM28 is incompetent to do without MAGEA3 or MAGEA6.

The interplay between autophagy and cancer is complicated. Early in cancer development, autophagy is thought

to be tumor suppressive. Later in cancer progression, autophagy is tumorigenic, where it allows tumors to survive during nutrient stress. Because of this dichotomy, we expect MAGEA3 and MAGEA6 to be activated early in tumor initiation by oncogenic insults. Consistent with this idea, previous reports have established that the bacterium *Helicobacter pylori* is able to induce the expression of MAGEA genes. This is especially intriguing because *H. pylori* promotes gastric cancer. Thus, *H. pylori* may reactivate MAGEA3 or MAGEA6 in order to downregulate autophagy to

avoid autophagic clearance and as a result it promotes gastric cancer. Furthermore, reduction in autophagy by MAGEA-TRIM28 may also play a role in lung cancer development. Previous publications have established that MAGEA3 is induced by smoking and is present in smokers who do not yet show signs of disease. These examples suggest that the early activation of MAGEA3 or MAGEA6 and ensuing downregulation of autophagy may be important factors in tumor initiation.

While the pathological significance of MAGEA3 and MAGEA6 is becoming clear, the normal physiological functions of MAGEA3 and MAGEA6 have not been elucidated. Unpublished results from our lab suggest that MAGEA3 and MAGEA6 are specifically expressed when maturing spermatocytes are undergoing a change in their energy source as they pass through the blood-testis-barrier. This point in spermatogenesis is critical as spermatocytes switch their carbon source from glucose derived from the blood stream to lactate derived from the supporting Sertoli cells. We hypothesize that one reason for MAGEA3 and MAGEA6 expression at this point in germ cell differentiation is that these proteins may protect cells from the consequence of prolonged energy stress and high levels of AMPK activation as cells transition across the blood-testis-barrier and switch carbon sources. However, additional roles for MAGEA3- and MAGEA6-mediated downregulation of AMPK during spermatogenesis are likely, including the critical regulation of the sterol synthesis pathway during spermatogenesis.

In summary, our study provides a novel regulatory mechanism by which cancer cells hijack a germline program that downregulates autophagy and promotes tumorigenesis. Given the widespread reactivation of MAGEA3 and MAGEA6 in many tumors, downregulation of the tumor suppressive AMPK pathway may not be restricted to only a subset of tumors with *STK11/LKB1* mutation as previously thought. Ultimately, identification of AMPK as a target of the MAGEA-TRIM28 ubiquitin

ligase may open new avenues for the use of AMPK-activating compounds in cancer treatment, specifically utilizing *MAGEA3* and *MAGEA6* expression status as an enrollment biomarker. Finally, our findings potentially illuminate a currently underappreciated interplay of AMPK

and autophagy in the process of spermatogenesis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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