Dab2 regulates autophagy: New insights into mechanisms of chemo-resistance and metastasis

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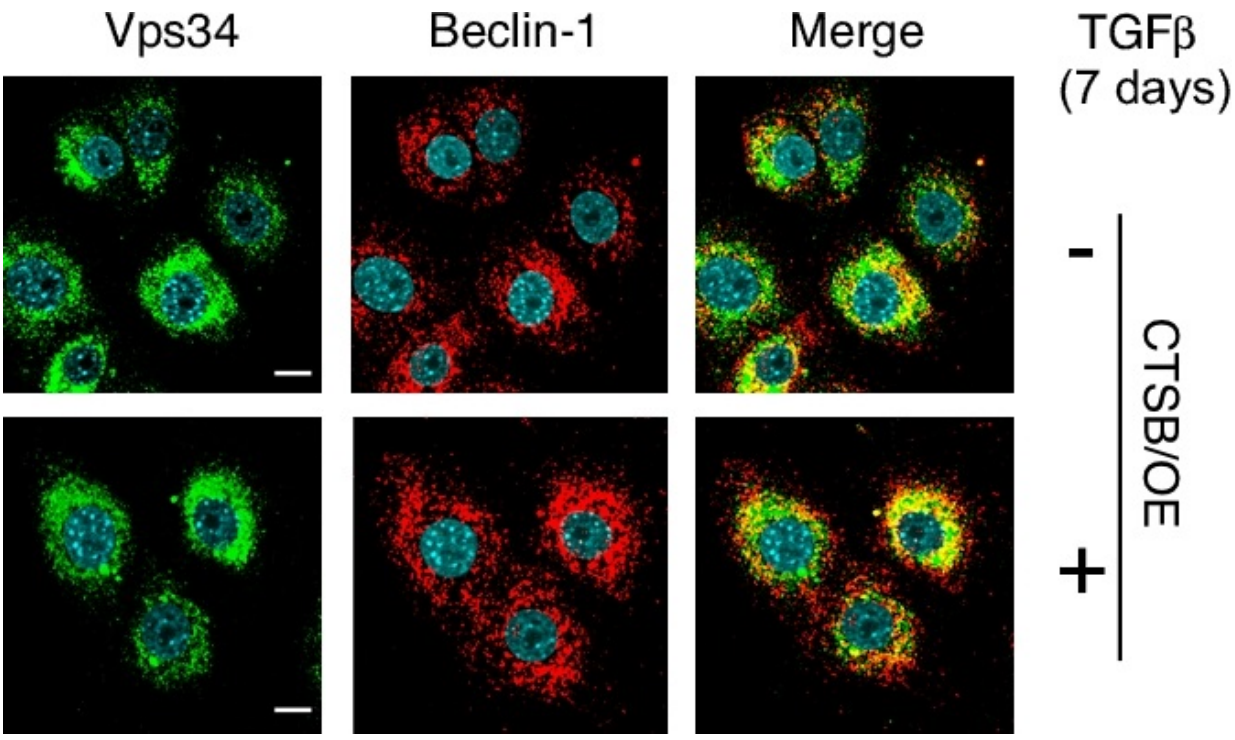

Immunofluorescence analysis to detect the expression and localization of Vps34 and Beclin-1 in cathepsin B overexpressing mouse mammary epithelial (CTSB/OE cells) treated -/+ TGF-beta? for 7 days. Photos were taken by confocal microscope. Scale bars, 10 m. The data show that in the absence of Dab2, due to CTSB overexpression, Vsp34/Beclin-1 interactions are maintained and autophagy is initiated. Credit: Adapted from a figure originally published online ahead of print on July 11, 2016 in *Nature Cell Biology* (doi: 10.1038/ncb3388) in an article by Jiang Y, Woosley AN, Sivalingam N.

Natarajan S, and Howe PH.

The results of preclinical studies by investigators at the Medical University of South Carolina (MUSC) reported in an article published online on July 11, 2016 in *Nature Cell Biology* demonstrate that disabled 2 (Dab2) serves as a molecular switch that regulates whether a tumor cell undergoes autophagy or apoptosis.

While expression of Dab2—an endocytic adaptor and tumor suppressor—is known to occur during transforming growth factor-beta (TGF-beta)-mediated epithelial-mesenchymal transition (EMT), the mechanisms by which it regulates apoptosis were, until now, poorly understood.

Exploring the pathways by which Dab2 is degraded and the effects of maintaining Dab2 levels reveals its pivotal role in preventing tumor cell survival by blocking autophagy and promoting cell death. These insights provide important information for maximizing the efficacy of existing chemotherapeutic agents.

TGF-beta induces EMT—a process by which cells transform from a polarized epithelial phenotype to a fibroblastic or mesenchymal one. Dab2 is expressed during TGF-beta-mediated EMT. While EMT is essential for normal cellular growth and homeostasis, it is abnormally activated in tumor cells and contributes to their chemo-resistance and metastasis.

TGF-beta has also been reported to regulate autophagy, which, in established tumors, ensures tumor cell survival through times of stress, as for example during chemotherapy. In other words, autophagy supports the chemo-resistance, growth, and metastasis of tumor cells.

Researchers focused on the Dab2 protein after noticing that, in cells treated with TGF-beta, Dab2 levels rose over the initial 24-48 hours as they went through EMT but then fell with continued TGF-beta? treatment. By day 7, the tumor cells had transitioned to a morphological state suggestive of either autophagy or apoptosis. Furthermore, the mesenchymal markers N-cadherin and vimentin, which like Dab2 initially rose during EMT, began to decline with longer exposure to TGF-beta.

"This was an unexpected finding that we followed," explains senior author Philip Howe, Ph.D., Professor and Chair of Biochemistry and Molecular Biology and Hans and Helen Koebig Chair in Clinical Oncology at MUSC. "We knew that if you give cells TGF-beta, they go through EMT, and we knew you needed Dab2 for TGF-beta-mediated EMT. But, when we kept adding TGF-beta for more sustained periods (after EMT took place), cells took on a different morphology and we noticed a loss of Dab2. We investigated this loss of Dab2 and discovered that it was being cleaved and that the cells were undergoing autophagy. Upon sustained TGF-beta treatment, the cells lost their mesenchymal phenotype they'd gained in EMT and entered into an autophagic state."

The team began to explore how prolonged TGF-beta treatment led to loss of Dab2 and the mesenchymal phenotype.

First, they found that longer TGF-beta exposure significantly increased cathepsin B (CTSB) expression and promoted its co-localization with Dab2. The team then not only demonstrated that CTSB is responsible for cleaving Dab2 but also that it recognizes the cleavage site by the flanking amino acids Val499 and Gly500. Thus, while an unaltered Dab2 sequence (Leu-Val-Gly-Leu) was degraded by CTSB, it did not cleave a mutant Dab2 sequence (Leu-Val-Leu).

Second, findings showed that, after 7 days, continuous TGF-beta?

treatment induced autophagy and down-regulated markers of apoptosis. This was particularly notable because these conditions promote tumor cell chemo-resistance and metastasis.

Third, they found that CTSB inhibition or expression of a mutant Dab2 without the CTSB cleavage site (i.e., the Leu-Val-Leu mutant) led to time-dependent increases in pro-apoptotic markers. When TGF-beta was withdrawn, cells in which Dab2 had been preserved underwent cell death. This series of experiments show not only how Dab2 is modulated by CTSB but also that it serves as a switch for regulating TGF-beta-induced autophagy and apoptosis.

Another series of experiments was undertaken to clarify exactly how Dab2 functions to prevent autophagy and promote apoptosis. These findings show that Dab2 inhibits TGF-beta-induced autophagy by blocking the Vps-Beclin-1 interaction and promotes apoptosis by attenuating ERK-Bim interactions.

Finally, the team used the chemotherapeutic agent doxorubicin (DOXO) to determine whether the role of Dab2 in inhibiting autophagy might affect tumor cell chemo-sensitivity. They found that cells in which CTSB was overexpressed had increased survival in the presence of DOXO. However, cells with high Dab2 levels due to CTSB inhibition or expression of the CTSB-resistant Dab2 mutant were more chemo-sensitive and underwent apoptotic changes. Thus, Dab2 was shown to promote chemotherapeutic drug-induced cell death by attenuating drug-induced autophagy. In vivo tumor studies in mice further found that Dab2 both enhanced DOXO-mediated cell death and attenuated tumor cell metastasis.

These direct insights into molecular mechanisms supporting tumor cell survival and death are crucial for maximizing the effectiveness of existing chemotherapeutic agents. "This is important because there aren't

a whole lot of drugs out there," explains Howe. "Most of what we use today has been around for 20 or 30 years because of a lack of investment in basic science."

The team's next steps are to investigate in vivo models for combination therapies using DOXO and a CTSB inhibitor to further illuminate the potential for targeting Dab2 as a means of reducing tumor recurrence and metastasis.

More information: Cathepsin-B-mediated cleavage of Disabled-2 regulates TGF-beta-induced autophagy, *Nature Cell Biology*, DOI: 10.1038/ncb3388

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