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Autophagy-dependent expression of osteopontin and its downstream Stat3 signaling contributes to lymphatic malformation progression to lymphangiosarcoma

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ABSTRACT

Lymphatic malformation (LM) is a vascular anomaly from lymphatic endothelial cells (ECs), and a fraction of the patients could progress to the deadly malignant lymphangiosarcoma (LAS). Using genetic tools to delete an essential autophagy gene Rb1cc1/FIP200 or its mutation specifically blocking its autophagy function, we demonstrated that autophagy inhibition abrogated LM progression to LAS although not affecting LM formation in our recently developed mouse model of LAS. Analysis of the mouse models in vivo and vascular tumor cells in vitro showed that autophagy inhibition reduced vascular tumor cell proliferation in vitro and tumorigenicity in vivo without affecting mTORC1 signaling as an oncogenic driver directly. Transcriptional profiling of autophagy-deficient tumor cells and further mechanistic studies revealed a role for osteopontin (OPN) and its downstream Jak/Stat3 signaling in mediating regulation of vascular tumor cells by autophagy. Together, these results support potential new prophylactic strategies to targeting autophagy and/or its downstream OPN expression to prevent progression of the benign LM to the malignant and deadly LAS.

Abbreviations: LM: lymphatic malformation; EC: endothelial cell; LAS: lymphangiosarcoma; OPN: osteopontin; RB1CC1: RB1 Inducible Coiled-Coil 1; FIP200: FAK family-interacting protein of 200 kDa.

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Lymphangiosarcoma; lymphatic malformation; endothelial cells; autophagy; RB1CC1/FIP200; Osteopontin; Stat3

Endothelial cells (ECs) in the adults are normally quiescent, but dysfunction in various signaling pathways in ECs could lead to a wide range of vascular anomalies, including vascular malformation and tumors. Lymphatic malformation (LM) and lymphangiosarcoma (LAS) are vascular anomalies originating from lymphatic ECs. While LM mostly remains a benign disease, and vascular anomalies in infants often stabilize and regress spontaneously, a fraction of LM patients will progress to LAS, a highly aggressive tumor currently without effective treatment and a high mortality. Although LM has been recognized as a risk factor for the deadly LAS, the underlying mechanisms regulating the progression of LM to LAS is not well understood, in part due to the lack of appropriate animal models for the disease until recently.

Autophagy plays complex roles in tumor cells as well as stromal cells in the tumor microenvironment to impact on tumorigenesis and progression. Paradoxical observations of both pro-tumorigenic and tumor suppressive functions of various autophagy genes remain a major challenge in the field, likely due to autophagy regulation of many cellular functions in different contexts as well as that many autophagy genes may possess non-canonical autophagy functions. Thus, observed effects in the autophagy gene knockout studies of various mouse models of cancer could be caused by the loss of their non-canonical functions or the combination of both autophagy and non-canonical functions. Our lab has taken a rigorous genetic approach to address this challenge by generating and analyzing the effects of either genetically ablating the essential autophagy gene *Rb1cc1* (also called *Fip200*) (i.e., 2cKO mice) or genetically disrupted its autophagy function (i.e., 2cKI mice) in different mouse models of cancer. In this study, we combined these genetic tools and our recently developed mouse model ($Tsc1^{i\Delta EC}$ mice) of human LAS that exhibits LM formation and progression to LAS to demonstrated that autophagy blockade by deleting *Rb1cc1* or specifically disrupting its autophagy function, while not affecting LM development, abolished LM progression to LAS in vivo [1].

To facilitating mechanistic studies of autophagy in vascular tumor cells, we employed two independent new vascular tumor cells derived from our $Tsc1^{i\Delta EC}$ model and showed that knockdown or knockout of *Rb1cc1* or other autophagy genes such as *Atg5* and *Atg7* decreased their proliferation and colony formation in vitro and tumorigenicity in xenografts. These results provide further support that autophagy is required for tumorigenicity of vascular tumor cells. Importantly, no difference in mTORC1 activation was detected in Rb1cc1-deficient tumor cells in vitro or xenograft tumors despite previous reports that autophagy could affect mTORC1 activation in other *Tsc1* knockout cells. Similarly, mTORC1 hyper-activation was not affected in 2cKO mice in vivo as measured by *Ulk1* phosphorylation status, excluding the possibility that autophagy inhibition blocked LM progression to LAS by directly reducing the hyper-activation of mTORC1 signaling as an oncogenic driver in $Tsc1^{i\Delta EC}$ model and vascular tumor cells from the model.

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To understand the underlying mechanisms of blockade of LM progression to LAS by autophagy inhibition, we carried out transcriptional profiling of *Rb1cc1* KO, *Atg5* KO, and *Atg7* KO vascular tumor cells in comparison to control cells to search for common changes in gene expression and signaling pathways in all three cells upon autophagy blockade by ablation of different autophagy genes. Such common targets are more likely changed by autophagy inhibition rather than the loss of non-canonical functions of these genes (likely to be different among these three cells). Analysis of these results revealed significant changes of PI3K-Akt signaling, ECM-receptor interaction and TNF signaling pathways in all three KO cells, which are likely to contribute to the altered functions of vascular tumor cells upon autophagy inhibition as well as the defective LM progression to LAS in 2cKO mice. Among a small set of genes present in all three pathways and altered in all three KO cells, we then focused on the potential role of *Spp1* gene encoding Osteopontin (OPN), a multifunctional protein that regulates tumor cell proliferation, survival, and migration, and is implicated in promoting invasive and metastatic progression of many cancers.

Using both vascular tumor cells *in vitro* and samples from 2cKO and control $Tsc1^{i\Delta EC}$ mice *in vivo*, we validated down-regulation of *Spp1* gene and OPN protein upon autophagy inhibition via different ways. We further showed that autophagy-dependent expression of OPN and its autocrine stimulation of Jak/Stat3 signaling are key factors in promoting vascular tumor cell proliferation and tumorigenicity by autophagy. In support of this mechanism, we observed that autophagy inhibition by deletion of *Rb1cc1* and other autophagy genes reduced expression of OPN and Jak/Stat3 signaling, and conversely, re-expression of Rb1cc1 or OPN rescued OPN levels and Jak/Stat3 signaling as well as defective proliferation and tumorigenicity of Rb1cc1-null vascular tumor cells. Nevertheless, as a multifunctional protein, OPN has been shown to affect several other signaling pathways besides Jak/Stat3 signaling. It also exists in several isoforms that have intracellular roles to regulate various cell functions by its different isoforms in addition to acting as an extracellular ligand through binding to cell surface receptors such as integrins and CD44. It will be interesting to further evaluate potential contributions of other downstream pathways as well as its intracellular vs extra-cellular functions to mediate the regulation of vascular tumor cells by autophagy in future studies. Likewise, it will also be interesting to examine potential contributions of other genes in the PI3K-Akt signaling, ECM-receptor interaction and/or TNF signaling based on transcriptional profiling upon as well as synergy among them in the block of LM progression to LAS by autophagy inhibition in future studies.

Autophagy inhibition is increasingly recognized as a potential treatment for various cancers, but current observations of both pro-tumorigenic and tumor suppressive functions of various autophagy genes present significant barriers for future progress. However, our studies provide more support that inhibiting autophagy could potentially prevent progression of the benign LM to the malignant and deadly LAS. More specifically, given that LM is a risk factor for LAS and the lack of effective treatment for the deadly LAS, such potential new prophylactic strategies to targeting autophagy and/or its downstream OPN expression will have important clinical implications for LM patients.

Disclosure statement

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Reference

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