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AUTHOR'S VIEW

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Tumor suppression by control of matrix metalloproteinase recycling

Marte Sneeggen, Kay O. Schink, and Harald Stenmark

Centre for Cancer Cell Reprogramming, Institute of Clinical Medicine, University of Oslo, Montebello, Norway; Department of Molecular Cell Biology, Institute for Cancer Research, Oslo University Hospital, Montebello, Norway

ABSTRACT

Secretion of matrix metalloproteinases (MMPs) enables cancer cells to degrade extracellular matrix, thus promoting tumor invasion and metastasis. We have recently found that the endosomal protein WDFY2 serves as a gatekeeper for MMP recycling from endosomes and that deletion of WDFY2, which is frequently lost in metastatic cancers, causes increased matrix degradation and cell invasion.

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Metastasis, the most life-threatening aspect of cancer, is initiated by local invasion of cancer cells into the tissue that surrounds the tumor.[1] Secretion of matrix metalloproteinases (MMPs) facilitates this process by degrading the extracellular matrix that otherwise serves as a barrier to the invading cancer cells.[2] MMPs can be secreted from several intracellular compartments, which include Golgi-derived secretory vesicles, late endosomes, or recycling vesicles derived from early endosomes.[3,4] The latter is an important secretory mechanism for membrane-anchored MMPs such as membrane-type 1 MMP (MT1-MMP, also known as MMP14) because such MMPs are endocytosed after being exposed at the cell surface.[5] Nevertheless, the mechanisms that control endocytic recycling of MMPs have remained largely unknown.

We recently identified a regulator of MT1-MMP recycling from endosomes, WD40- (W/D-containing repeat of 40 amino acids) and FYVE (conserved in Fab1, YOTB, Vps27, and EEA1)-domain-containing protein 2 (WDFY2), which is found on endosomes.[6] The seven WD40 domains of WDFY2 are predicted to form the blades of a β-propeller,[7] whereas the C-terminal FYVE domain of WDFY2, like other FYVE domains, binds to the membrane lipid, phosphatidylinositol 3-phosphate (PtdIns3P). The latter interaction is required for WDFY2´s localization to endosomes.[6]

Using confocal fluorescence microscopy, we observed that WDFY2 is mainly found on endosomes positive for early endosomal antigen 1 (EEA1). However, WDFY2 and EEA1 localize to distinct domains of these endosomes. EEA1 is known to localize mainly to the vacuolar part of early endosomes, whereas live and super-resolution microscopy revealed that WDFY2 accumulates at the base of endosomal tubules. Further analyses of these tubules showed that they belong to the actin-stabilized class of endosomal tubules implicated in slow endocytic recycling, as opposed to the actin-negative tubules involved in bulk recycling.[8]

Having found that PtdIns3P is required for localization of WDFY2 to endosomes, we asked whether the WDFY2-containing tubules are enriched for this lipid. However, using a standard PtdIns3P probe, a tandem FYVE domain of the endosomal protein, hepatocyte growth factor-regulated tyrosine kinase substrate (HRS) (2xFYVE^{Hrs}), we observed only weak labeling of the WDFY2-containing tubules. We, therefore, made a similar construct consisting of a tandem FYVE domain of WDFY2 (2xFYVE^{WDFY2}), and this probe strongly labeled endosomal tubules while showing weaker labeling of the vacuolar part of the endosome. This selectivity can probably be explained by the preference of the WDFY2 FYVE domain for highly curved PtdIns3P-containing membranes, as we detected in vitro. We conclude that WDFY2-containing tubules are rich in PtdIns3P and that the FYVE domain of WDFY2 is specialized for detecting this PtdIns3P pool.

A proteomic approach revealed the vesicle-associated membrane protein 3 (VAMP3) as an interaction partner of WDFY2, and this interaction was confirmed biochemically. The interaction with VAMP3 is interesting because VAMP3 is known to mediate fusion of endosome-derived vesicles with the plasma membrane.[4,9] Surprisingly, when we measured the extent of fusion of VAMP3-containing vesicles with the plasma membrane, we observed a marked increase of such fusion events in WDFY2 knockout cells. This indicates that WDFY2 is a negative regulator of VAMP3 recycling.

One of the known cargoes of VAMP3-containing vesicles is MT1-MMP,[4] and we, therefore, examined whether MT1-MMP recycling is controlled by WDFY2. Indeed, MT1-MMP was found to co-localize with WDFY2 and VAMP3 on early endosomes, and knockout of WDFY2 resulted in increased exocytosis of MT1-MMP. In contrast, recycling of transferrin receptors, which is known to occur from actin-negative endosome tubules, was not affected. From this, we conclude that

CONTACT Kay O. Schink kay.oliver.schink@rr-research.no; Harald Stenmark stenmark@ulrik.uio.no Centre for Cancer Cell Reprogramming, Institute of Clinical Medicine, University of Oslo, Montebello, Oslo N-0379, Norway

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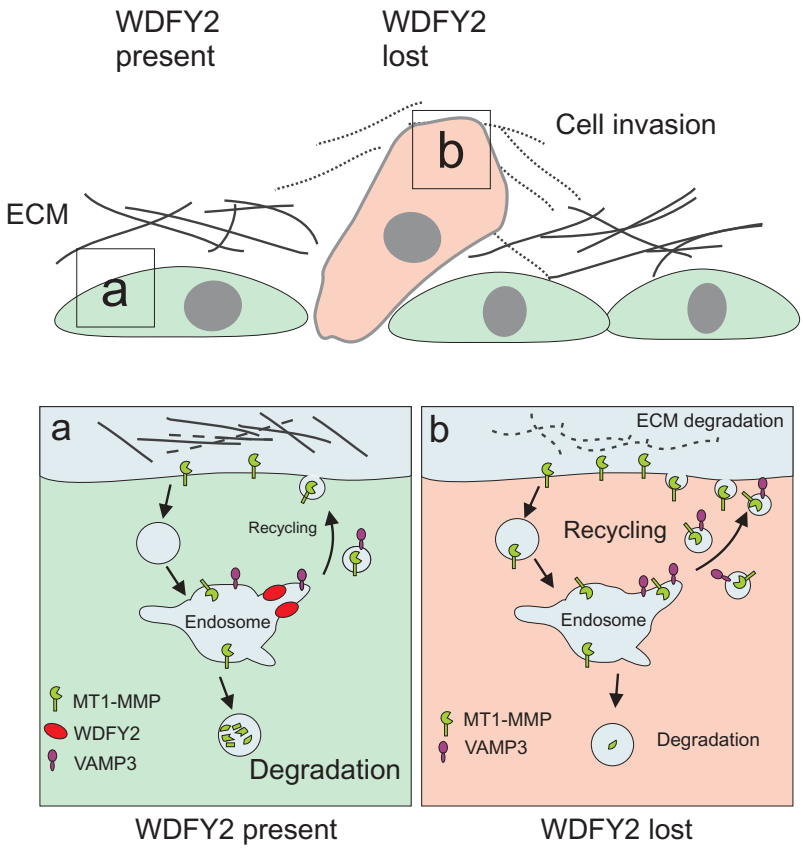

Figure 1. Control of MT1-MMP recycling by WDFY2. (a) In cells expressing WDFY2, the formation of vesicle-associated membrane protein 3 (VAMP3)-positive membrane-type 1 metalloproteinase (MT1-MMP)-containing recycling vesicles is constrained, which limits the surface expression of MT1-MMP and extracellular matrix (ECM) degradation. (b) In contrast, loss of WDFY causes enhanced secretion of MT1-MMP-containing vesicles. The lack of WDFY2 leads to increased formation and exocytosis of VAMP3-dependent recycling vesicles, causing increased recycling and membrane delivery of MT1-MMP. This leads to enhanced ECM degradation and invasivity.

WDFY2 specifically controls endocytic recycling of MT1-MMP on VAMP3-containing vesicles.

In theory, increased secretion of MT1-MMP should lead to increased degradation of the extracellular matrix, and we indeed found a strong VAMP3-dependent increase in extracellular matrix degradation in WDFY2 knockout cells. This degradation could be blocked by an MMP inhibitor or by knockdown of MT1-MMP, showing that WDFY2-regulated secretion of MT1-MMP by VAMP3-containing vesicles does control the ability of the cell to degrade extracellular matrix.

The next question was then whether WDFY2 also controls cell invasion through the extracellular matrix. Interestingly, knockout of WDFY2 in noninvasive retinal pigment epithelial cells converted these cells to a strongly invasive phenotype both in Matrigel and collagen 3D matrices. Likewise, invasive breast cancer cells became even more invasive upon siRNA-mediated depletion of WDFY2. Conversely, when WDFY2 was reintroduced into invasive prostate cancer cells that have very low endogenous expression of WDFY2, the invasivity of these cells was lost. We conclude from these experiments that WDFY2 controls the invasivity of both normal and malignant cells.

These findings beg the question whether WDFY2 is a tumor suppressor. WDFY2 is frequently deleted in metastatic ovarian and prostate cancers,[6] and it is interesting to note that a cyclin-dependent kinase inhibitor 2D (*CDKN2D*)-*WDFY2* fusion gene occurs in 20% of all high-grade serous ovarian cancers.[10] This gene fusion results in a truncated and presumably dysfunctional WDFY2 protein. While the definitive answer will have to await studies in animal models, we think there is sufficient evidence to propose that WDFY2 is a tumor suppressor and that it acts via a novel mechanism, namely by restraining MMP recycling from endosomes (Figure 1). Through its interaction with VAMP3, WDFY2 restricts the budding of MT1-MMP-containing VAMP3 vesicles from actin-stabilized endosomal tubules. Upon deletion of WDFY2, this negative control is lost, allowing faster recycling of MT1-MMP to the plasma membrane for increased matrix degradation and cell invasion.

Disclosure of potential conflicts of interest

No potential conflict of interest was reported by the authors.

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