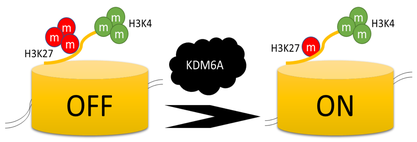
KDM6A, Lysine Demethylase, Loss Affects Epithelial-to-Mesenchymal Related Gene Expression and Golgi Apparatus Morphology

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Epigenetic modifications such as histone modifications regulate gene expression



KDM6A is a histone demethylase specific for H3K27me3. Removal of two methyl groups from H3K27me3 allows for gene expression

EMT drives progressive but reversible loss of KDM6A protein, not transcript

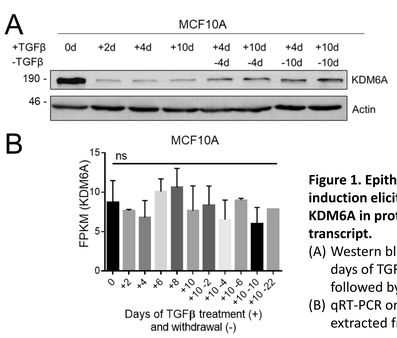


Figure 1. Epithelial-to-mesenchymal induction elicits a reversible loss of KDM6A in protein amount but not in transcript.
(A) Western blot of MCF10A cells after 10 days of TGFβ (5ng/mL) induced EMT followed by no EMT for 10 days.
(B) qRT-PCR on triplicate samples of RNA extracted from cells as in (A)

Inhibition or silencing of KDM6A lead to increases in migration and mammosphere formation

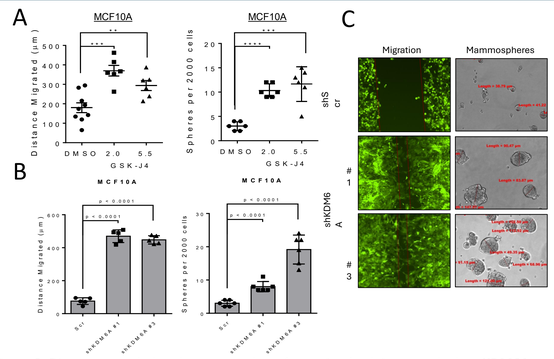


Figure 2. Distance traveled and mammosphere formation both increased when KDM6A was inhibited with GSKJ4 or silenced.
(A) MCF10A cells were treated with either 2.0μM or 5.5μM of GSK-J4 and subjected to a scratch assay and analyzed for migration. Chemical inhibition of KDM6A increased distance migrated.
(B) MCF10A cells had KDM6A silenced and were subjected to a scratch assay and analyzed for migration. Silencing of KDM6A increased distanced migrated.
(C) Visual analysis of cell migration and mammospheres formed when KDM6A was silenced.

Reversibly suppressed KDM6A is necessary for maintaining epithelial identity

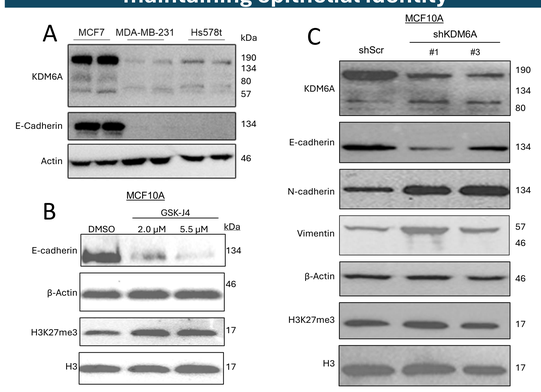


Figure 3. KDM6A is expressed in greater amounts in epithelial cells. Chemical inhibition or silencing of KDM6A elicits suppression of E-cadherin protein and increase in N-cadherin protein.
(A) Western blot of whole cell lysate from MCF7, MDA-MB-231, and Hs578t cells showing differences in KDM6A and E-cadherin protein expression dependent on epithelial or mesenchymal cell state.
(B) Western blot of MCF10A cells treated with either control DMSO, 2.0μM GSKJ4, or 5.5μM GSK-J4. When KDM6A is inhibited by GSK-J4, E-cadherin protein is decreased and H3K27me3 is increased.
(C) Western blot of MCF10A cells with silenced or controlled KDM6A protein expression. E-cadherin expression is increased alongside with a decrease in N-cadherin.

KDM6A protein is present in the membranous cellular fraction with WDR5 and ASH2L

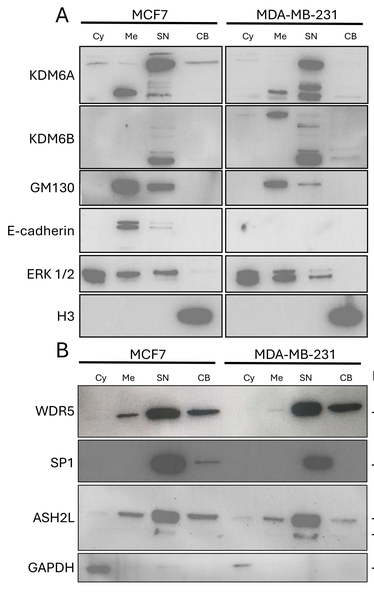


Figure 4. Fractioned MCF7 and MDA-MB-231 cells display KDM6A and compass-like complex proteins WDR5 and ASH2L in the membranous fraction.
(A-B) Western blot of MCF7 and MDA-MB-231 cells protein fractions showing localization of KDM6A and other members of the compass –like protein complex.
(C) Peptide count (confidence score) of compass-like complex members in the cytoplasmic, membranous, and soluble nuclear fractions of MCF7 cells.

C

	CY	ME	SN
KDM6A	53 (230)	59 (307)	96 (522)
WDR5	17 (84.5)	17 (86.4)	90 (597)
ASH2L	44 (221)	n.d.	121 (782)
RBBP5	n.d.	n.d.	76 (421)
DPY30	n.d.	n.d.	21 (121)
KMT2C	14 (79.5)	n.d.	
KMT2D	n.d.	5 (30.1)	25 (144)

KDM6A Enrichment at the Golgi body corresponds to EMT state

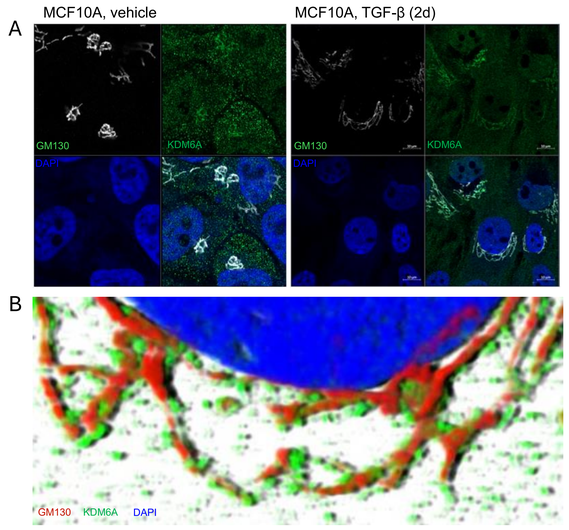


Figure 5. KDM6A is retained in the Golgi body but lost in the nucleus upon EMT induction.
(A) Immunofluorescence staining of MCF10A cells treated with vehicle or TGFβ for 2 days for GM130, KDM6A, and DAPI
(B) Enlarged image showing co-localization of KDM6A with GM130, a Golgi body marker

KDM6A Enrichment at the Golgi body corresponds to EMT state

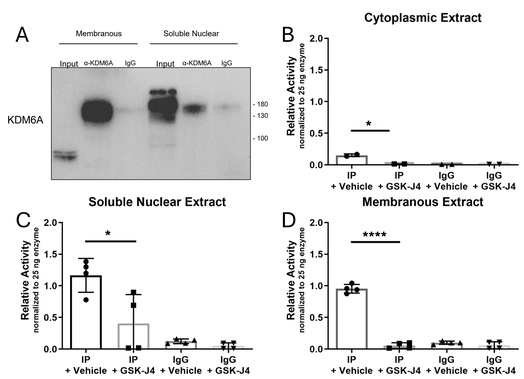


Figure 6. KDM6A proteins retain enzymatic activity in the membranous fraction.
(A) Western blot for KDM6A of membranous and soluble nuclear protein fractions of MCF7 cells for input, a-KDM6A IP, and control IgG
(B-D) Enzymatic activity assay for KDM6A in the cytoplasmic, soluble nuclear, and membranous factions respectively

Schematic of Nucleus-to-Golgi Distance Measurement

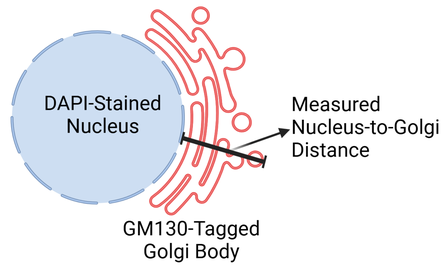


Figure 7. ImageJ was used to measure the perpendicular distance between the nucleus and the furthest Golgi body point in addition to the Golgi body area.

KDM6A maintains epithelial morphology

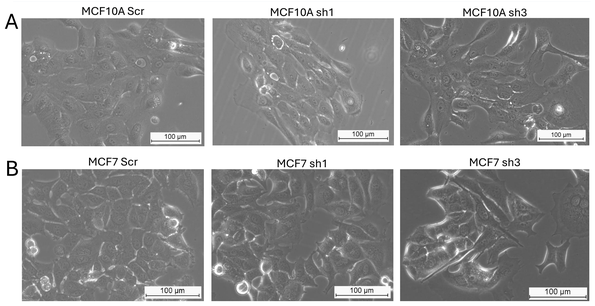


Figure 8. KDM6A KD MCF10A (A) and KDM6A KD MCF7 (B) brightfield images at 20X measured on a Nikon Eclipse Ts2R light microscope.

KDM6A is a regulator of Golgi body morphology

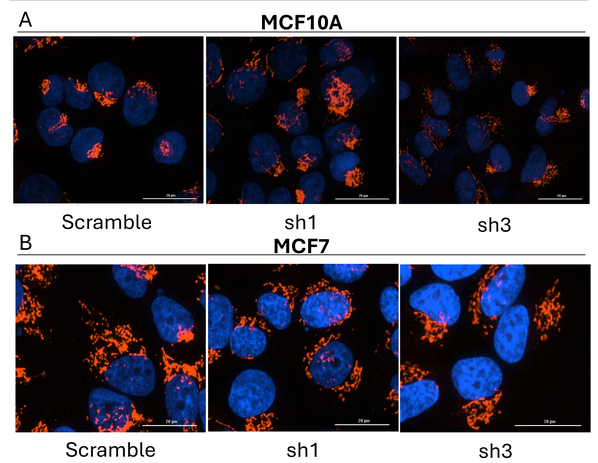


Figure 5. Golgi body morphology is altered upon knockdown of KDM6A in MCF10A and MCF7 cells.
(A) KDM6A was knocked down in MCF10A cells, stained with DAPI (Blue) and tagged with GM130 (Red) and imaged using a BioTek Cytation C10 Spinning-Disk Confocal Microscope at 60X magnification. Images obtained were derived from deconvoluted maximum intensity Z-stacks
(B) KDM6A was knocked down in MCF7 cells, stained with DAPI (Blue) and tagged with GM130 (Red) and imaged using a BioTek Cytation C10 Spinning-Disk Confocal Microscope at 60X magnification. Images obtained were derived from deconvoluted maximum intensity Z-stacks

TGFβ induced EMT alters Golgi body morphology in MCF10A cells

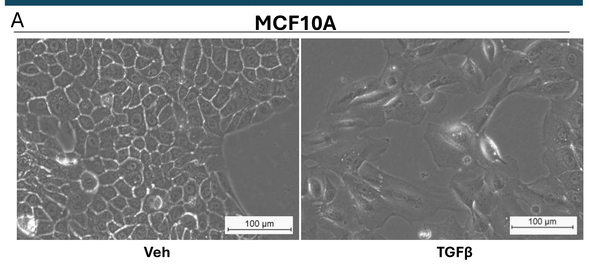


Figure 7. 10 day TGFβ treated MCF10A cells display changes of Golgi body morphology
MCF10A cells were treated with 5.0 ng/mL TGFβ and stained with DAPI (Blue) and tagged with GM130 (Red) and imaged using a BioTek Cytation C10 Spinning-Disk Confocal Microscope at 60X (A) to access Golgi morphology and at 20X measured on a Nikon Eclipse Ts2R light microscope (B) to access morphology. Confocal images obtained were derived from deconvoluted maximum intensity Z-stacks

TFGB-induced EMT and KDM6A alters Relative Golgi Area and Nucleus-to-Golgi Distance

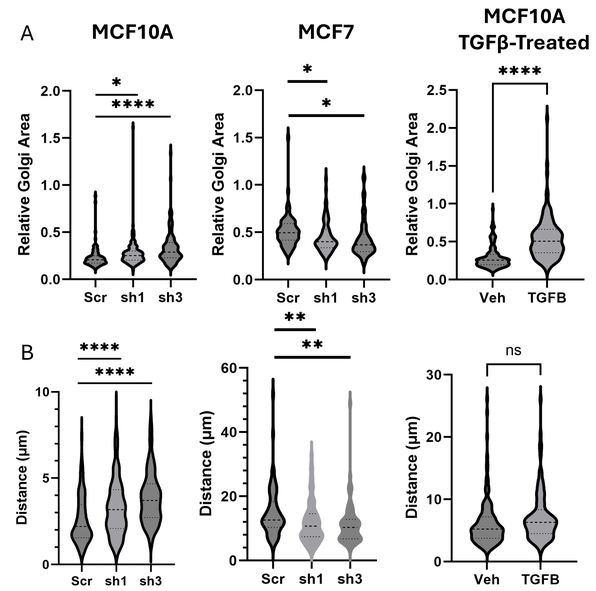


Figure 9. Relative Golgi Area (A) and Maximal perpendicular Nucleus-to-Golgi body distance (B) is altered upon KDM6A KD in MCF10A and MCF7 cells and upon TGFβ-induced EMT. Golgi body and nucleus localization was quantified using ImageJ. Normalized area fraction was determined by dividing Golgi area to nucleus area in the same cell (n=50-150 cells per group). Golgi and nucleus elements were determined using threshold (Li). ImageJ was used to measure the perpendicular distance between the nucleus and the furthest Golgi body point to determine changes in nuclear-to-Golgi distance

Conclusions

- While KDM6A canonically demethylates H3K27me3 in the nucleus, its localization in the membranous fraction, specifically its co-localization with the Golgi apparatus indicates a possible alternative role. This is further supported by the presence of its fellow compass-like protein complex in the membranous fraction.
- KDM6A is enzymatically active in not only the soluble nuclear fraction, but also in the membranous fraction.
- KDM6A KD in both MCF7 and MCF10A induces changes in cell and Golgi body morphology. Dispersal or compaction of Golgi cisternae might lead to dysregulation of its functions in sorting, processing, and trafficking proteins. This may lead to alterations in the secretome and impact the tumor microenvironment.
- Golgi morphology alteration upon KDM6A knockdown is context dependent as demonstrated in opposite morphological changes in epithelial mammary ER+ cells versus in normal epithelial mammary cells.

Acknowledgments

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