Small molecule inhibitors of ATP7A as novel therapies for cancer

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Abstract

Recent studies suggest that trace element copper (Cu) plays a key role in cancer progression. Cu-dependent signaling pathways in cancer cells are poorly understood and are being actively investigated. In a recently published PNAS paper, we demonstrated that ATP7A copper transporter promotes breast and lung tumor growth and spread in mice by activating pro-cancerous enzyme, lysyl oxidase (LOX). Therefore, we propose that blocking ATP7A using small molecule inhibitors could be a powerful approach in blocking cancer progression. We screened for inhibitors of ATP7A using a structure-based virtual screen of ATP7A-interacting drug-like compounds, resulting in identification of our lead compound, MKV3. We used microscale thermophoresis and standard enzyme activity assays to measure the binding affinity of MKV3 to ATP7A and test the effect of MKV3 on ATP7A-dependent LOX and tyrosinase enzymes. *In vitro* scratch and tumorigenesis assays were performed to test the effect of MKV3 on cancer cell migration and tumor growth. Microscale thermophoresis experiments revealed that MKV3 binds to ATP7A with nanomolar affinity and is a potent inhibitor of ATP7A-dependent LOX activity and cell-migration of 4T1 breast, LLC and A549 lung cancer cells. Moreover, MKV3 inhibited ATP7A-dependent tyrosinase activity in B16 melanoma cells and suppressed B16 tumorigenesis *in vivo.* In summary, these studies have identified a novel first-in-class high-affinity inhibitor of ATP7A and provide a framework to design MKV3 derivatives with improved therapeutic efficacy in mouse models of cancer. Our findings have the potential for a sustained and powerful impact in cancer therapy.

Introduction

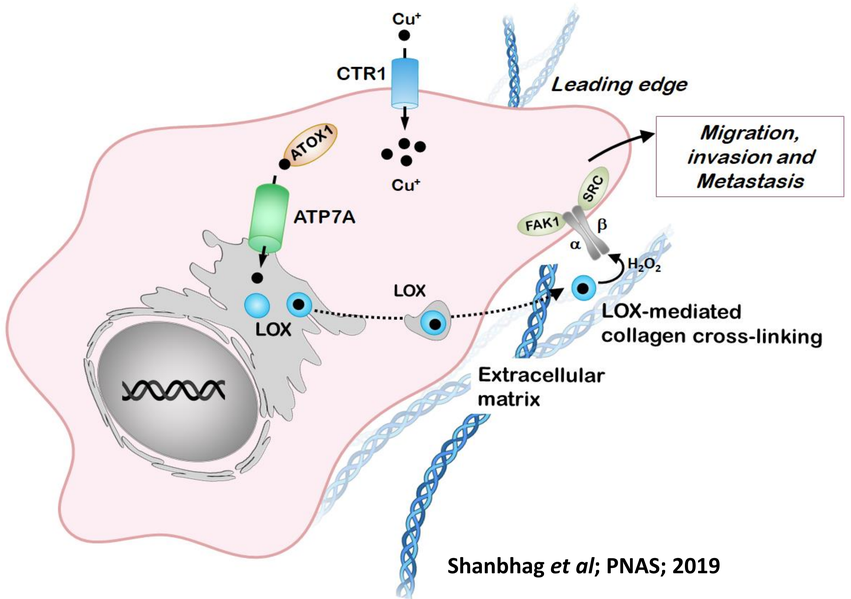

Fig.1. ***Schematic model of the role of ATP7A in LOX-mediated tumor metastasis.*** *Cu enters the cell through high affinity Cu importer (CTR1) and is transported to ATP7A via ATOX1 protein. The ATP7A Cu transporter delivers Cu into the lumen of the trans-Golgi network to metallate LOX protein, which plays key roles in tumor metastasis by collagen crosslinking and integrin mediated activation of focal adhesion kinase (FAK1), a key regulator of cell migration and invasion.*

The rationale for targeting ATP7A as a novel anticancer strategy – ATP7A belongs to the family of P1B type ATPases. It is ubiquitously expressed and is predominantly localized in the trans-Golgi network (TGN) where it serves two important functions. These include 1) copper export from cells which is coupled with ATP7A trafficking to the plasma membrane 2) copper transport into the Golgi complex to LOX enzyme. The most widely reported Cu-dependent pathway that contributes to cancer involves the LOX enzyme. Therefore, blocking the delivery of copper to the LOX enzyme represents an innovative and powerful strategy to block LOX-dependent malignancies. The significance of our study is the innovative use of Cu transport inhibitors as novel chemotherapeutic agents. This study has the potential to pave the way for clinical studies with MKV3 or its derivatives.

Results

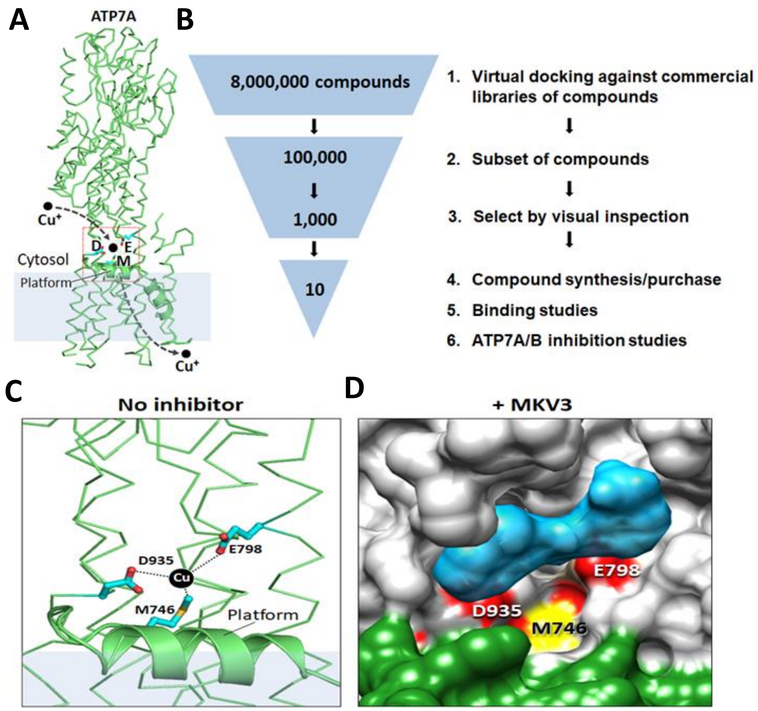

Fig. 2. ***A structural model of ATP7A highlighting putative Cu-binding triad of D, E and M amino acids near the platform and a pocket targeted for drug design. A)*** *The ATP7A protein (backbone view) highlighting the conserved triad of closely arranged Met-Glu-Asp at the mouth of the funnel.* ***B)*** *Pipeline for identification of inhibitors of ATP7A* ***C)*** *A close-up of this region showing Cu bound within the triad in a predicted trigonal planar configuration.* ***D)*** *A space-filling model highlighting the pocket and the location of our lead compound (MKV3in cyan) docked within the pocket.*

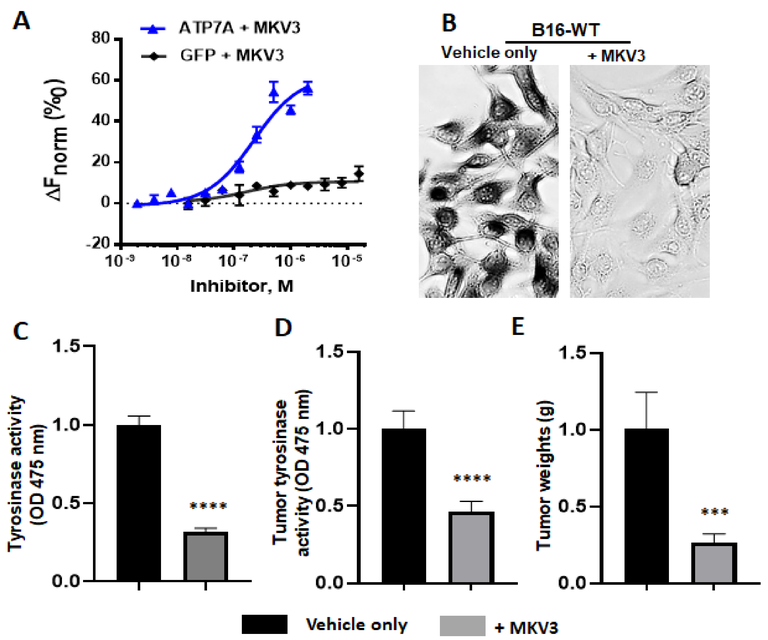

Fig. 3. ***MKV3 inhibits ATP7A activity in vitro and in vivo*** *(**A**) Analysis of MKV3 binding to ATP7A using microscale thermophoresis with normalized fluorescence (Fnorm) on y-axis and inhibitor concentration on x-axis .**B)** MKV3 inhibits tyrosinase activity in B16 melanoma cells. In-situ tyrosinase activity was measured in B16-WT cells treated with MKV3. Cells were treated with 5 µM MKV3or DMSO for 24 hrs and then tyrosinase activity was measured as the conversion of colorless L-DOPA to dopachrome. (**C**) Quantification of tyrosinase activity in B16-WT cells treated for 24 h with or without MKV3 (mean ± SEM; ****p < 0.0001). **(D and E)** Intratumoral injection of MKV3 in B16 melanoma tumor bearing C57/BL6 mice. Tumor tyrosinase activity and weights are shown (mean ± SEM; ***p < 0.001; ****p < 0.0001).*

Results

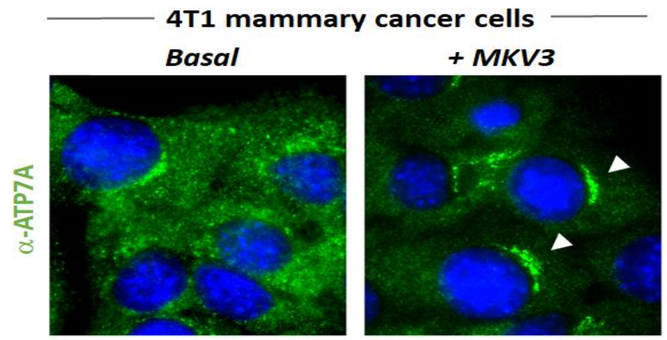

Fig. 4. ***MKV3 inhibits copper stimulated trafficking of ATP7A.*** *ATP7A protein is shown in green (Alexa488) and nuclei were labeled with DAPI (blue).*

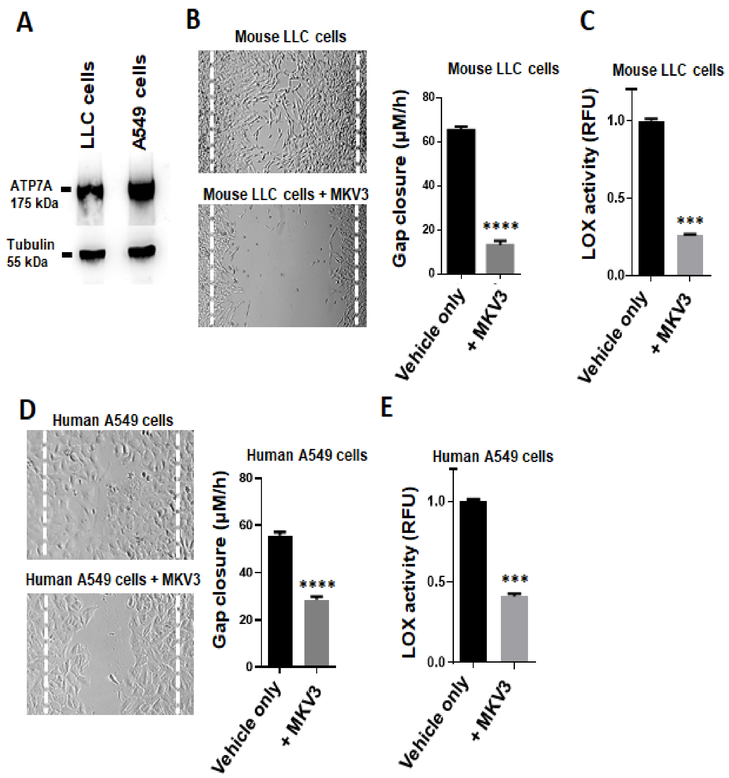

Fig. 5. MKV3 inhibits LOX activity and cancer cell migration (A) *Immunoblot analysis of ATP7A in LLC and A549 cells. (**B and D**) Invitro scratch assay demonstrating MKV3 reduces the motility of mouse LLC and human A549 lung cancer cells. Images show the extent of gap closure 24 h after scratch formation in the presence or absence of MKV3. Dashed lines indicate scratch starting points. Cell migration rate is shown (mean ± SEM; ****p < 0.0001). (**C and E**) LOX activity in the conditioned media of wild type mouse LLC and human A549 cells treated for 24 h with MKV3 or DMSO control (mean ± SEM; ***p < 0.001; RFU, relative fluorescence units).*

Summary

1. The discovery of the first inhibitor of any heavy metal ion transporter.
2. The innovative use of a Cu transport inhibitor to block the activity of LOX enzyme, cancer cell migration and tumorigenesis

Acknowledgements

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