World Congress on
Breast Cancer 2015

Downregulation of Ca^{2+}-activated Cl^- channel TMEM16A by histone deacetylase inhibition in breast cancer cells

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Breast Cancer-2015, Birmingham, UK
August 4, 2015

Overview

1. Role of Ca^{2+}-activated Cl^- channel in cancer cells and epigenetic modification of gene expression

2. Downregulation of Ca^{2+}-activated Cl^- channel, TMEM16A by a histone deacetylase (HDAC) inhibitor, vorinostat in human breast cancer cells

3. Effects of pharmacological and siRNA-based blockade of HDAC on TMEM16A transcription

4. Regulation of HER2 expression by TMEM16A

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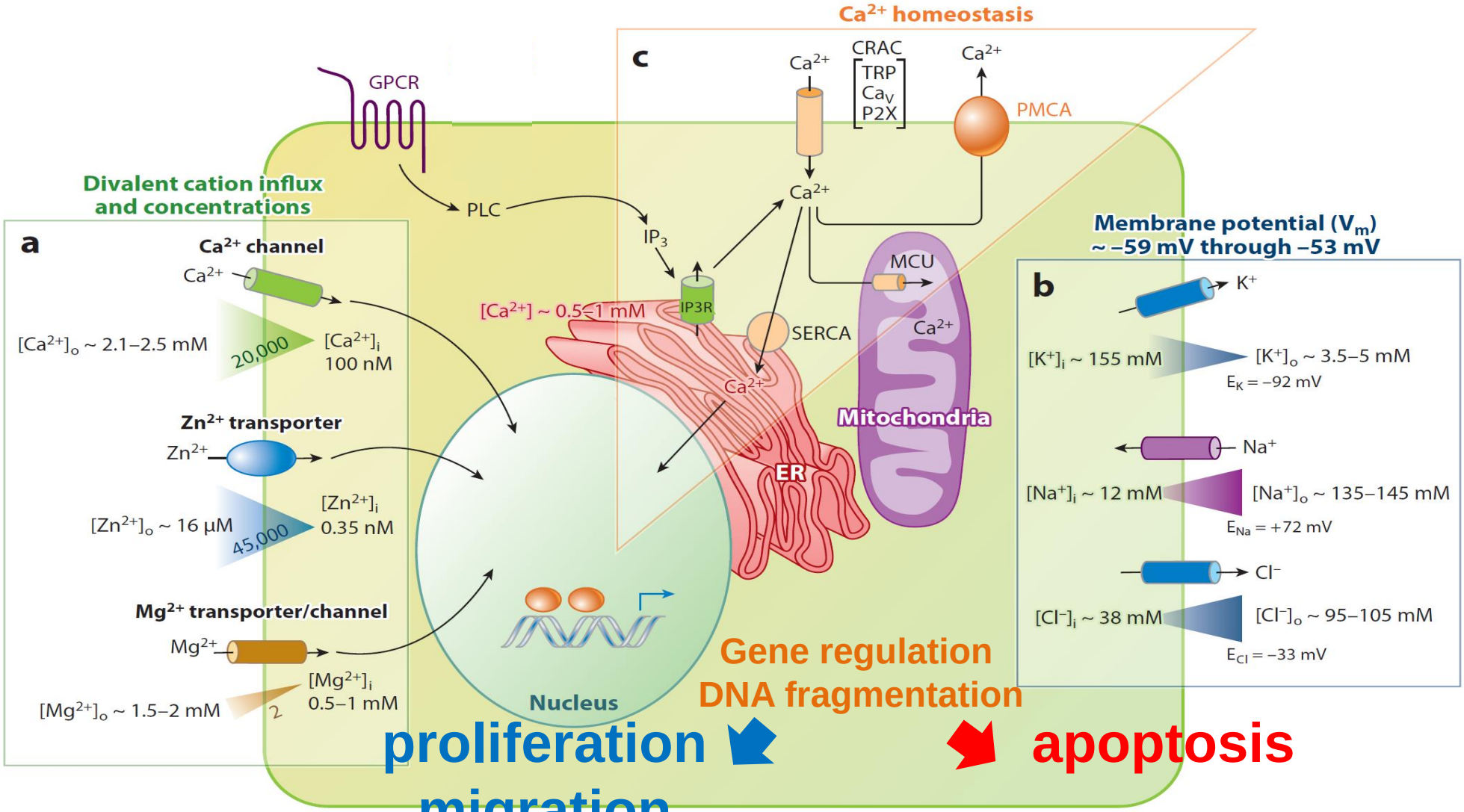
2. **Downregulation of Ca^{2+}-activated Cl^- channel, TMEM16A by a histone deacetylase (HDAC) inhibitor, vorinostat in human breast cancer cells**

3. **Effects of pharmacological and siRNA-based blockade of HDAC on TMEM16A transcription**

4. **Regulation of HER2 expression by TMEM16A**

Principal interplay between the Na^+-, K^+-, Ca^{2+}- and Cl^--permeable channels



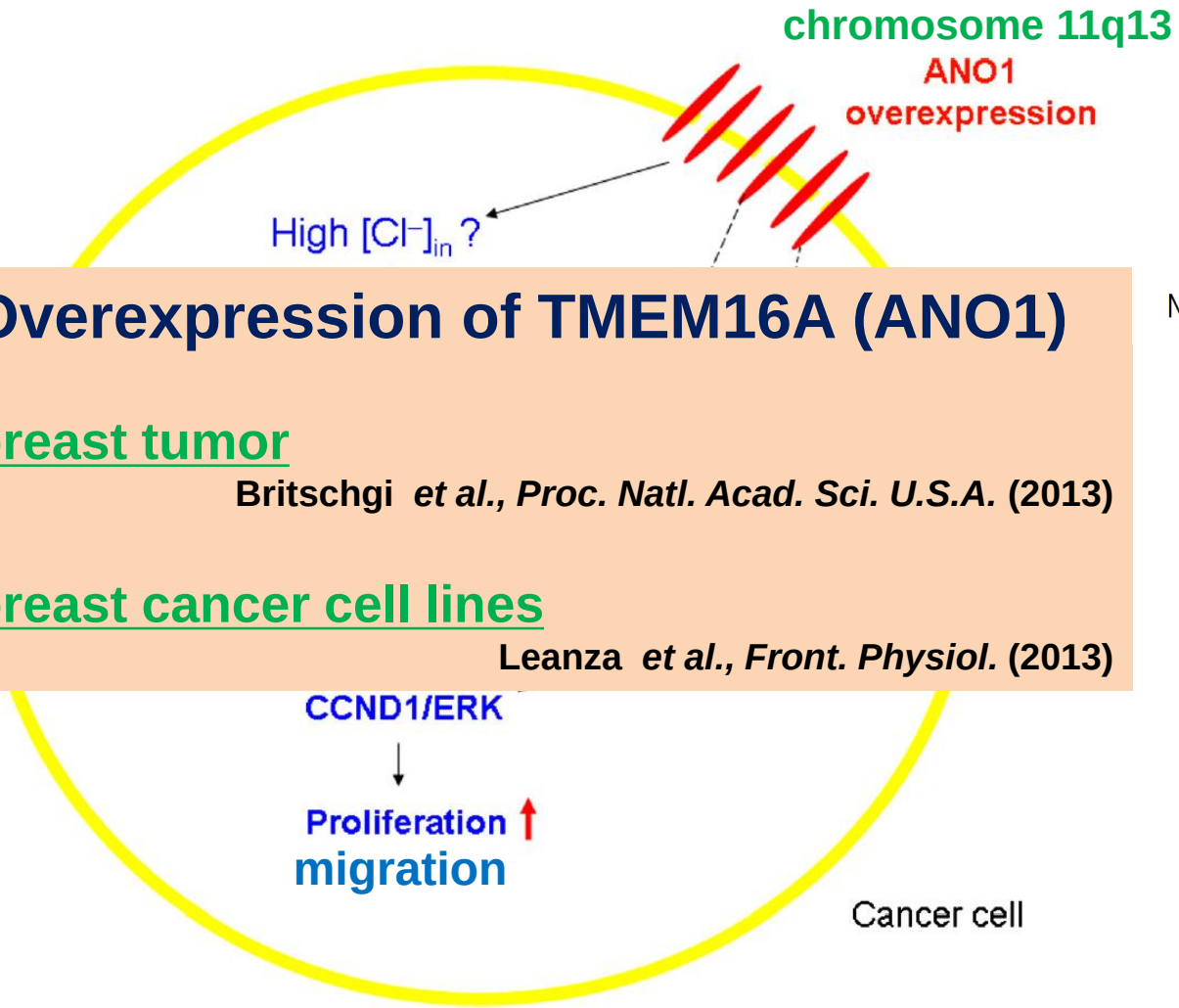
Feske *et al.*, *Ann. Rev. Immunol.*, 33, 291-353 (2015)

Ion channels and cancer

1. Ion channels contribute to various cancer processes by the regulation of the resting membrane potential and Ca^{2+} signaling.

2. Pharmacological inhibition of ion channels is an attractive target to prevent cancer cell proliferation and metastasis.

TMEM16A (ANO1) overexpression enhances cancer cell proliferation through signaling pathways



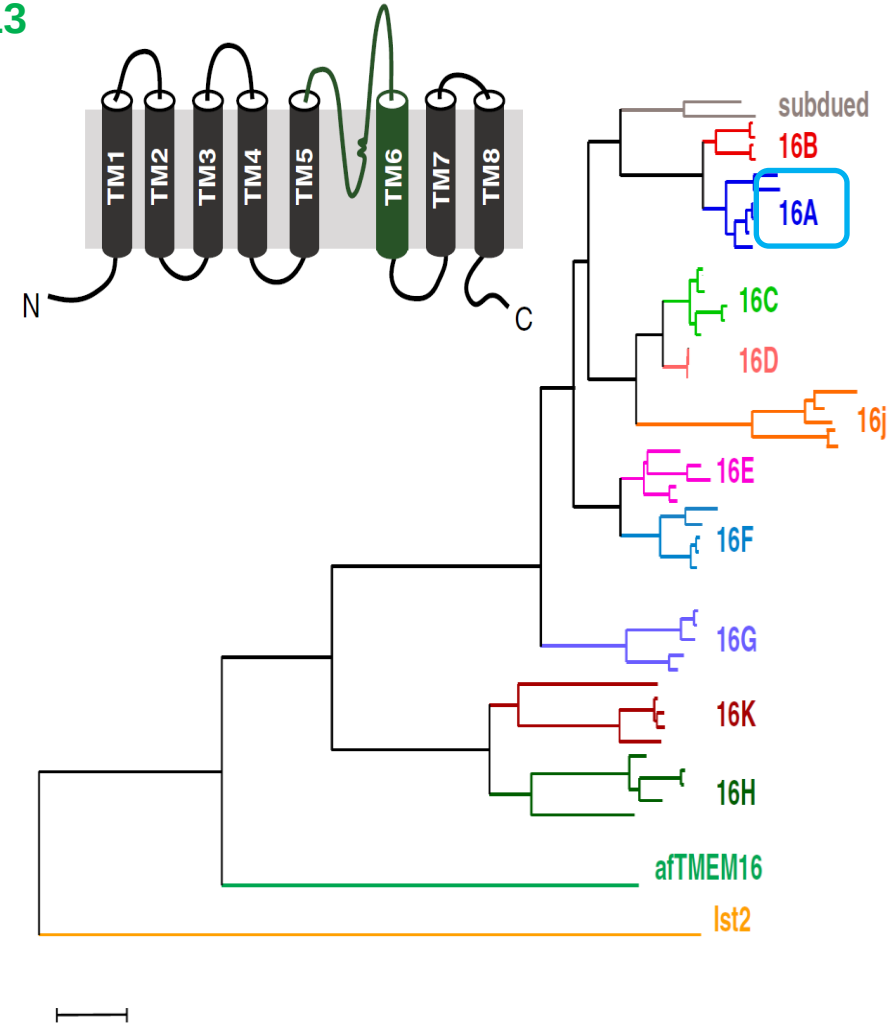
Overexpression of TMEM16A (ANO1)

breast tumor

Britschgi *et al., Proc. Natl. Acad. Sci. U.S.A.* (2013)

breast cancer cell lines

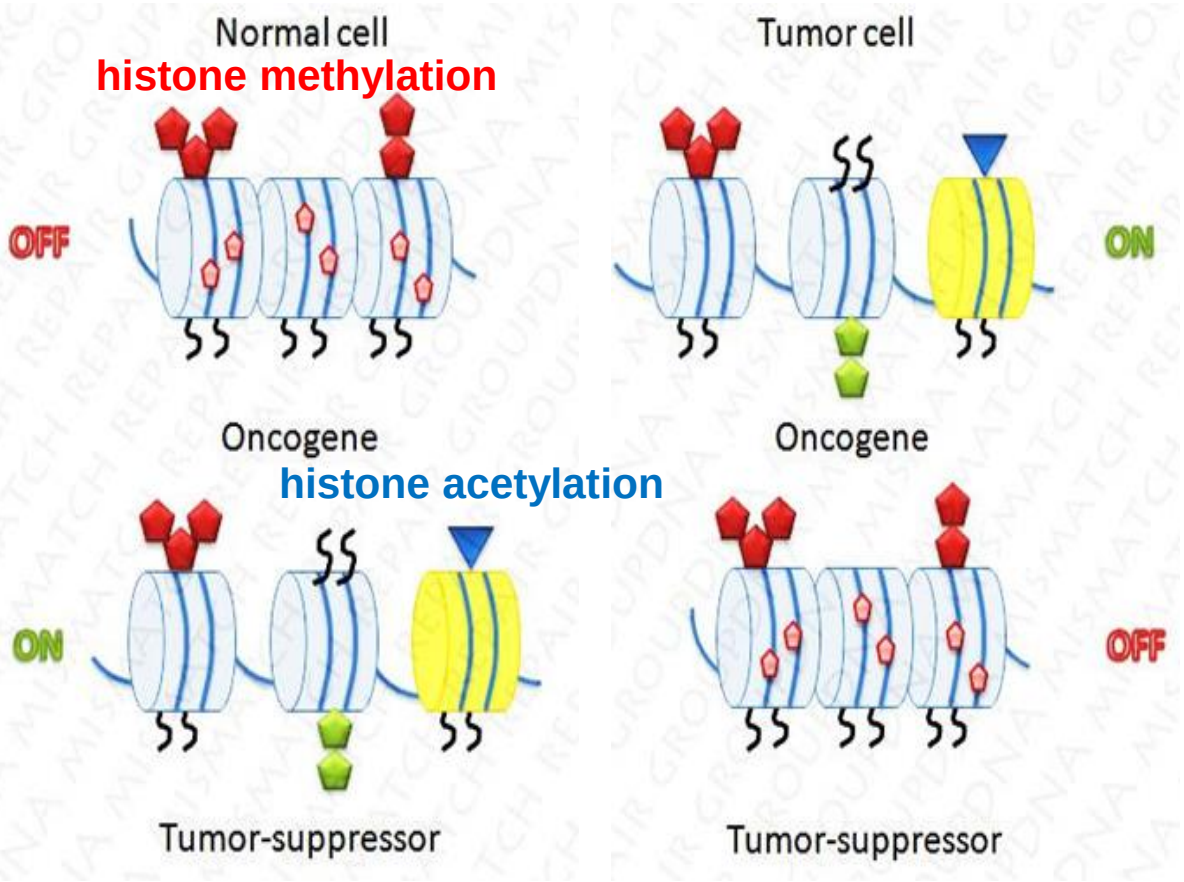
Leanza *et al., Front. Physiol.* (2013)



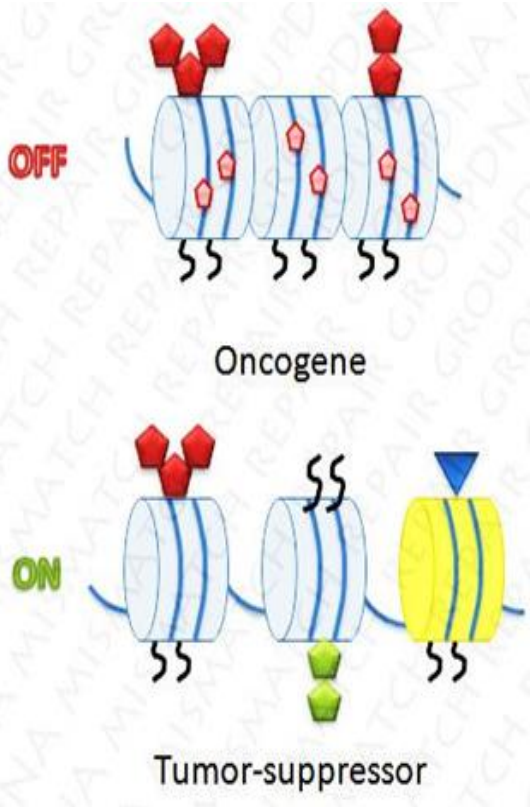
TMEM16A is an attractive therapeutic target and a novel biomarker for cancer.

Qu *et al.*, *Cancer Med.* 3, 453-461 (2014)
Picollo *et al.*, *J. Mol. Biol.* 427, 94-105 (2015)

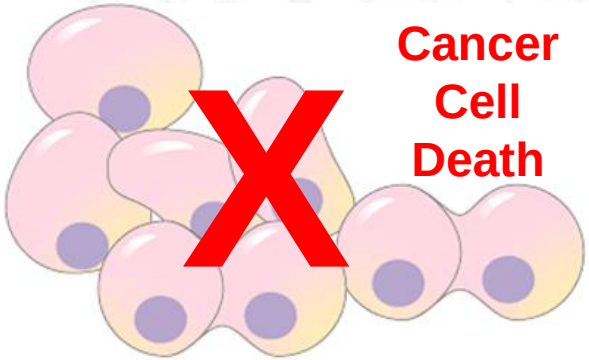
Epigenetic modification of gene expression



Histone Deacetylase Inhibitor



Epigenetic modification therapy with HDAC inhibitors is one of the novel strategies for cancer treatment.



Overview

1. Role of Ca^{2+}-activated Cl^- channel in cancer cells and epigenetic modification of gene expression

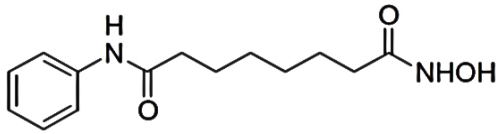
2. **Downregulation of Ca^{2+}-activated Cl^- channel, TMEM16A by a histone deacetylase (HDAC) inhibitor, vorinostat in human breast cancer cells**

Vorinostat is approved for the treatment of T cell lymphoma and is developed for the other solid tumors with combination drug therapy.

3. Effects of pharmacological and siRNA knockdown of HDAC on TMEM16A transcription

vorinostat **pan-HDAC inhibitor**

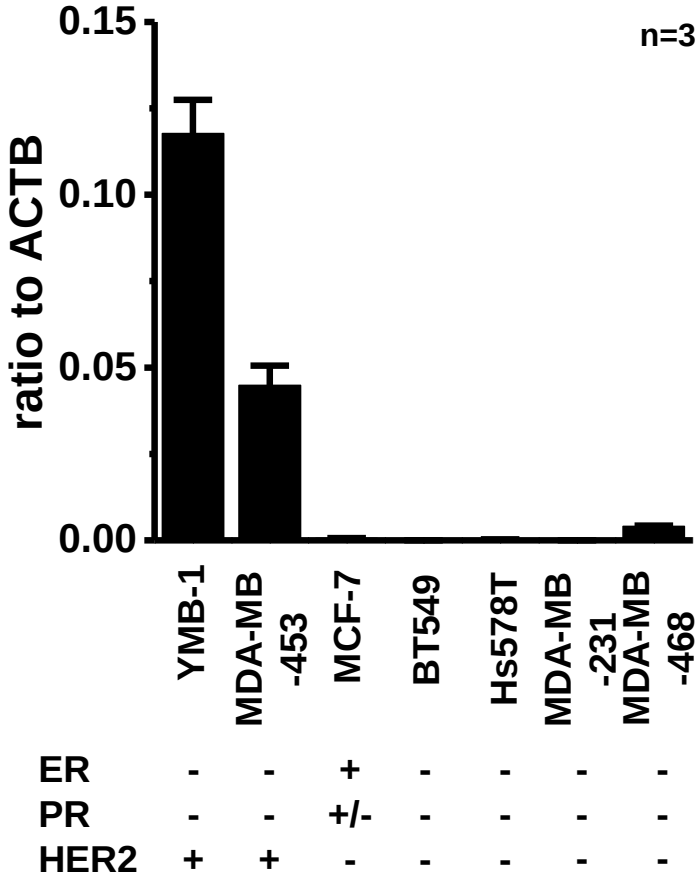
IC_{50}=100-1000 nM for HDAC1-11



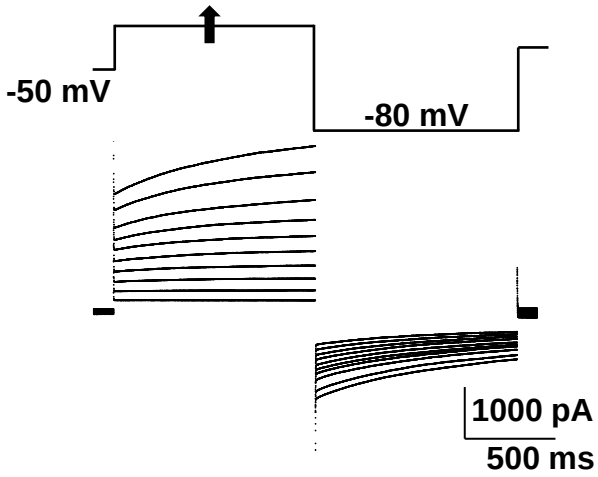
4. Regulation of HER2 expression by TMEM16A

Functional expression of TMEM16A in YMB-1 cells

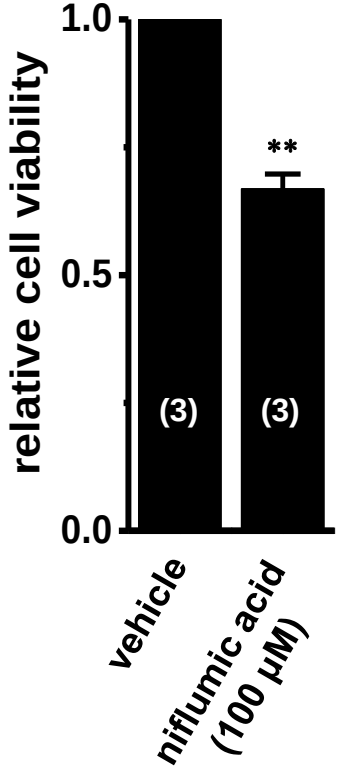
A. TMEM16A mRNA



B. TMEM16A activity in YMB-1



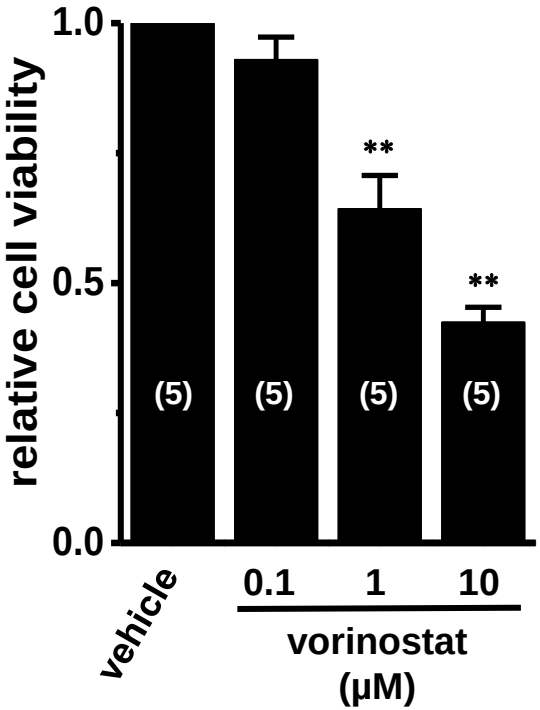
C. Cell viability



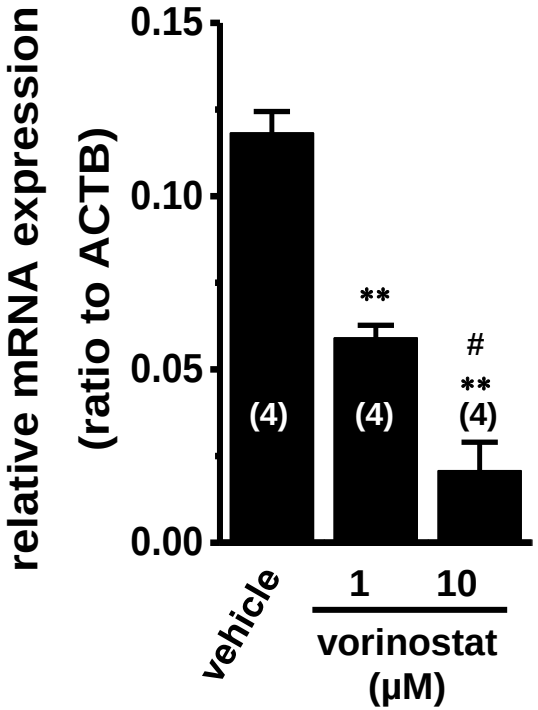
Matsuba *et al.*, *J. Pharmacol. Exp. Ther.*, 351, 510-518 (2014)

Downregulation of TMEM16A by treatment with vorinostat in YMB-1 cells (1)

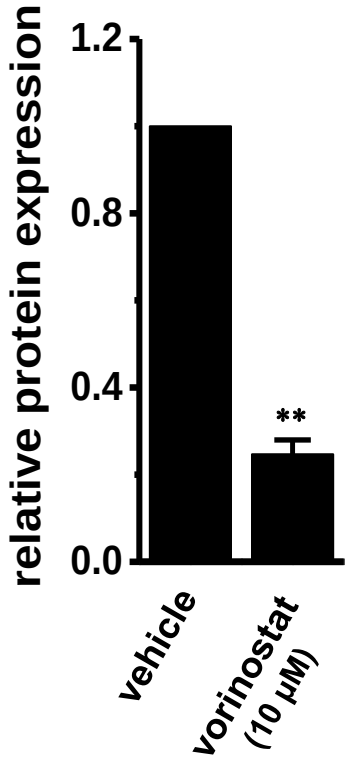
A. Cell viability

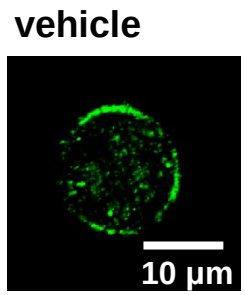


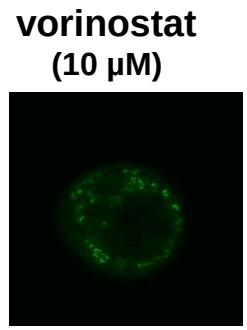
B. TMEM16A mRNA



C. TMEM16A protein

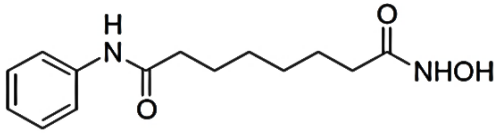






vorinostat pan-HDAC inhibitor

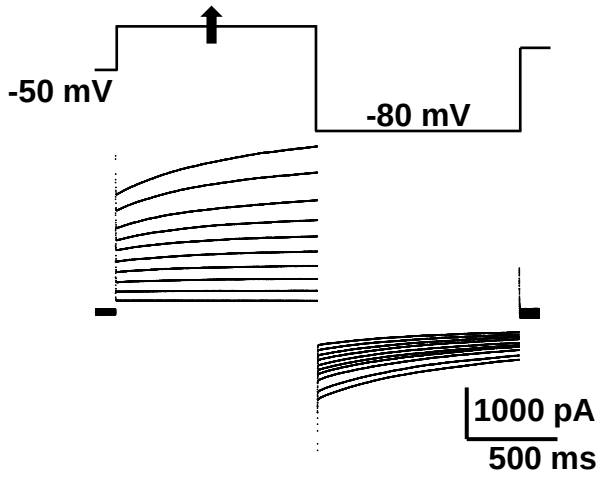
IC_{50}=100-1000 nM for HDAC1-11



Matsuba *et al.*, *J. Pharmacol. Exp. Ther.*, 351, 510-518 (2014)

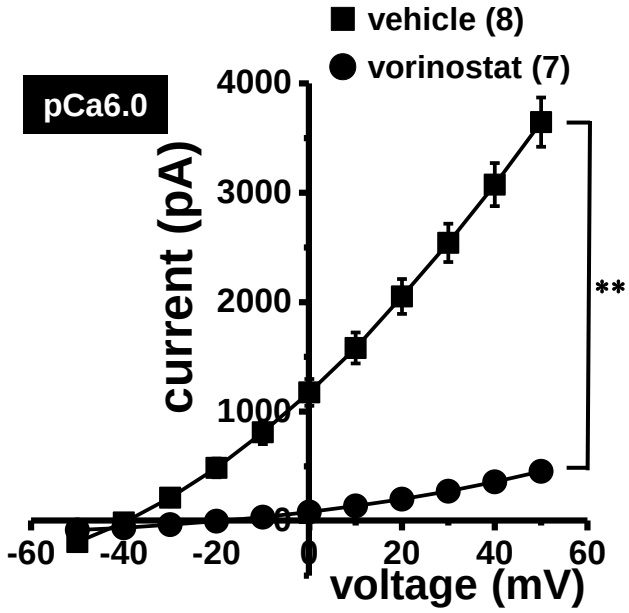
Downregulation of TMEM16A by treatment with vorinostat in YMB-1 cells (2)

A. vehicle



B. vorinostat (10 μM)

C. I-V relationship



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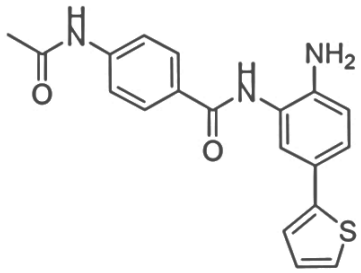
3. **Effects of pharmacological and siRNA-based blockade of HDAC on TMEM16A transcription**

   Of 11 HDAC subtypes, HDAC1, 2, 3 and 6 are highly expressed in YMB-1 cells in consistent with the expression patterns in human tumor breast tissues.

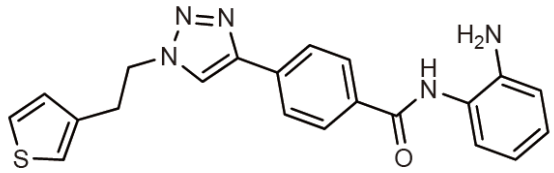
4. Regulation of HER2 expression by TMEM16A

Downregulation of TMEM16A by HDAC2/3 inhibition in YMB-1 cells (1)

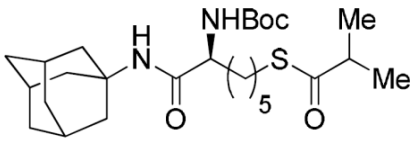
AATB HDAC1 & HDAC2 inhibitor
IC_{50}= 7 nM for HDAC1
IC_{50}= 49 nM for HDAC2



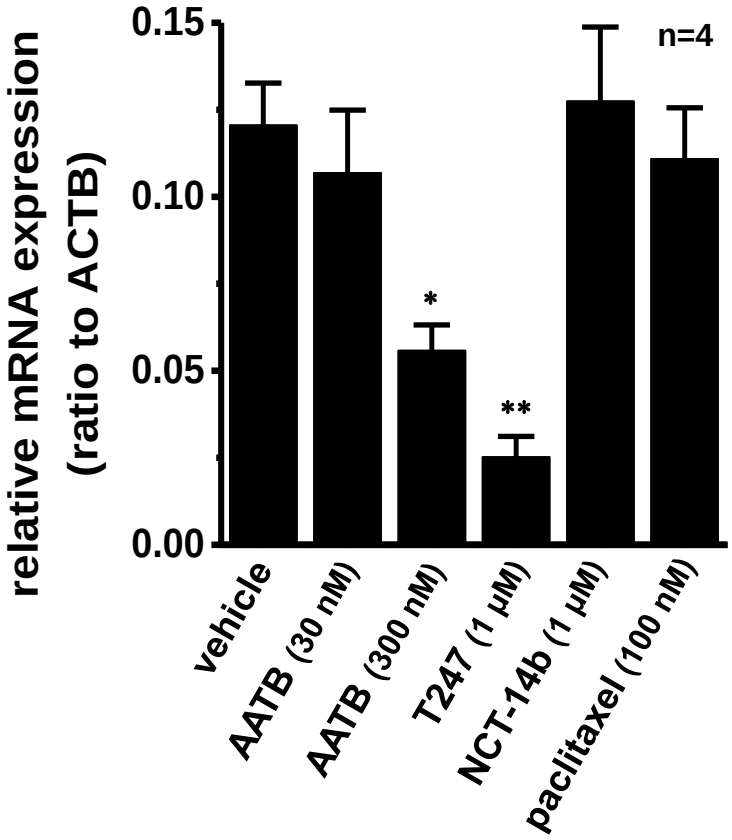
T247 HDAC3 inhibitor
IC_{50}= 240 nM for HDAC3



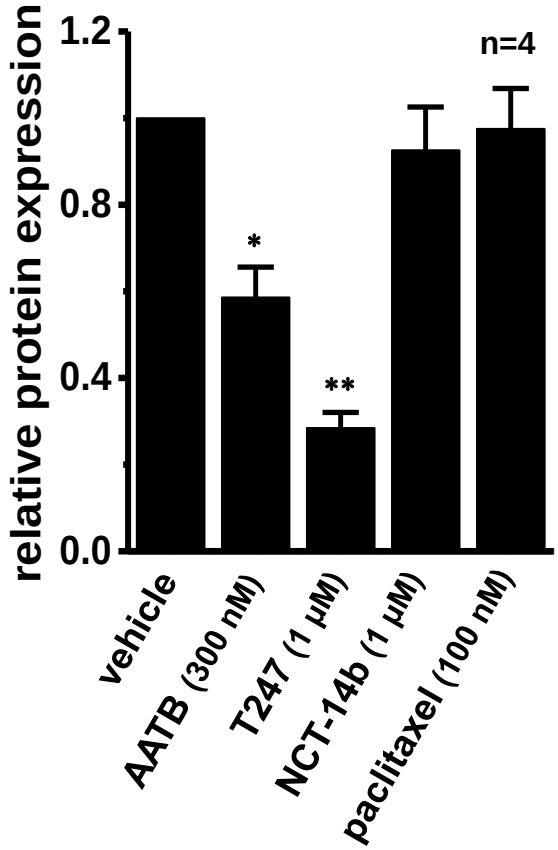
NCT-14b HDAC6 inhibitor
IC_{50}= 82 nM for HDAC6



A. TMEM16A mRNA



B. TMEM16A protein



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Downregulation of TMEM16A by HDAC2/3 inhibition in YMB-1 cells (2)

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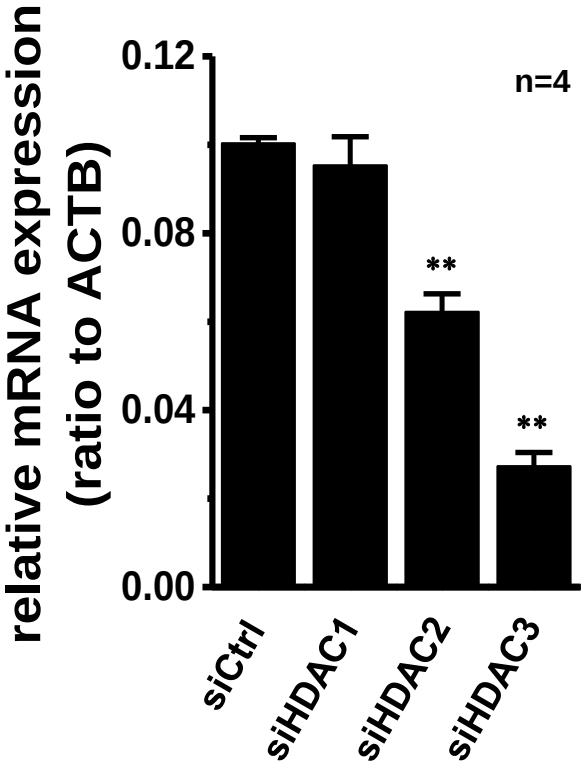
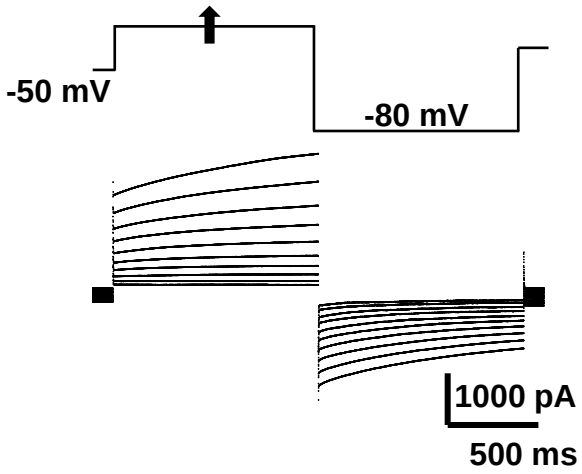
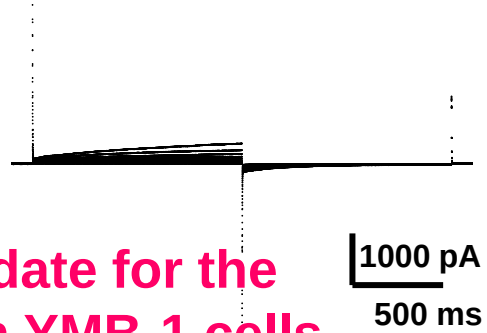


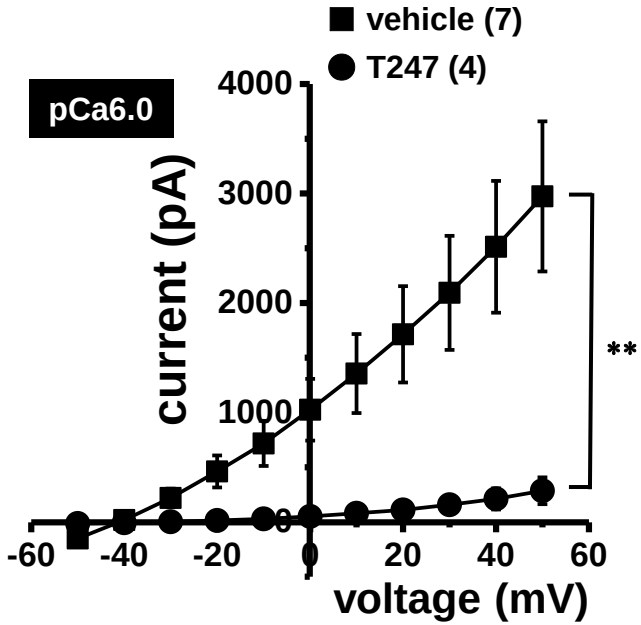
B. vehicle



C. T247 (1 μM)



D. I-V relationship



HDAC3 is the leading candidate for the downregulaion of TMEM16A in YMB-1 cells.

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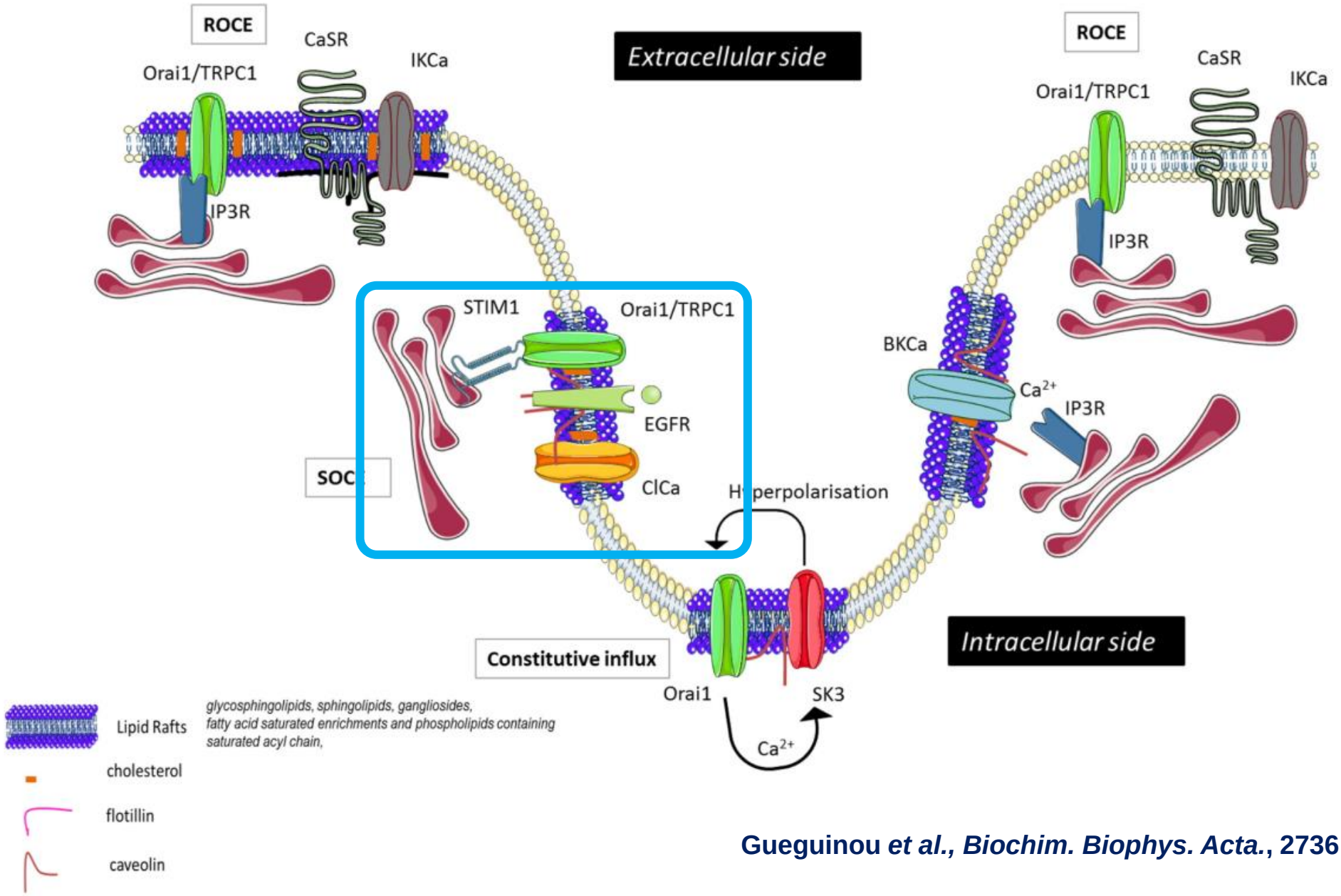
Lipid rafts, ion channel complexes and EGFR signaling

1) In the plasma membrane, ion channels and receptors can functionally communicate by forming clusters of lipid microdomains.

2) EGF receptor forms functional complexes with Ca^{2+}-activated Cl^- channel and store-operated Ca^{2+} channels and contributes to the Ca^{2+} signal pathway with them.

Lipid rafts, ion channel complexes and EGFR signaling

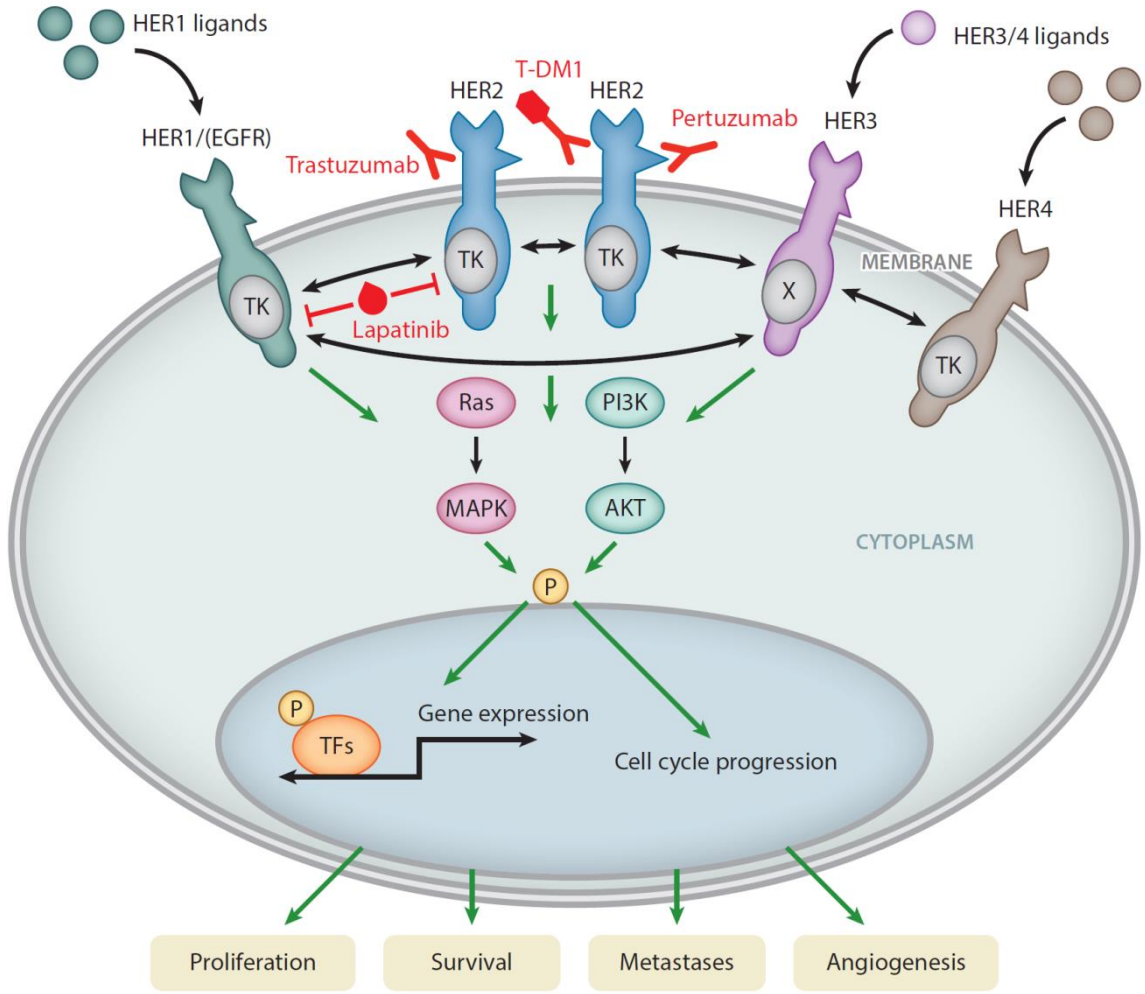


Gueguinou *et al., Biochim. Biophys. Acta.*, 2736 (2014)

HER signaling pathway and its related cell processes

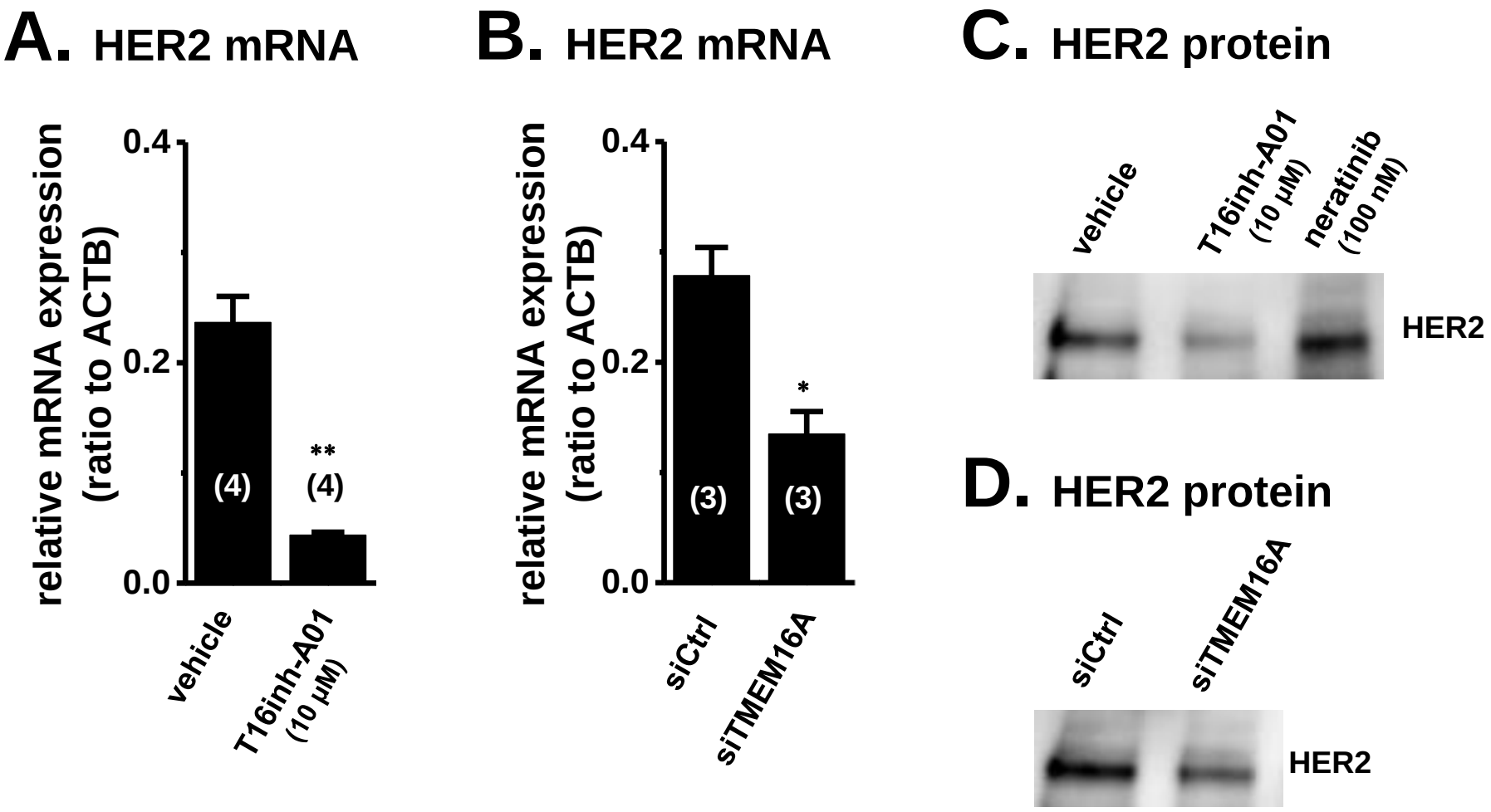
HER2 is

1) an EGF receptor family with tyrosine kinase activity.
2) overexpressed in about 30 % of patients with breast cancer.
3) the mainstay of targeted therapy for the treatment of invasive breast cancers.



Rimawi *et al., Annu. Rev. Med.*, 66, 111-128 (2015)

Downregulation of HER2 by TMEM16A inhibition in YMB-1 cells



Conclusions

1. Ca^{2+}-activated Cl^- channel, TMEM16A is a potential therapeutic target for breast cancer, and inhibition of TMEM16A suppresses proliferation and invasion.
2. In malignancies with a frequent gene amplification of TMEM16A, TMEM16A dysfunction by HDAC3 inhibition is at least in part responsible for the suppressive effects on cell viability in breast cancer cells, and it may be related to the poor invasion capacity observed in metastatic breast cancer cells.
3. TMEM16A may play an important role in the regulation of HER2-downstream signaling in breast cancer cells.

Acknowledgements

Kyoto Pharmaceutical University, Kyoto,Japan

Masanori Fujii, Ph.D.
Hiroaki Kito, Ph.D.
Satomi Niwa, Ph.D.

Sayo Matsuba
Saki Kanatsuka
Yurika Nakazono

Aichi Gakuin University, Nagoya, Japan

Electrophysiology
Katsuhiko Muraki, Ph.D.
Noriyuki Hatano, Ph.D.

Kyoto Prefectural University of Medicine, Kyoto Japan

Chemical synthesis of HDAC inhibitors
Takayoshi Suzuki, Ph.D.
Peng Zhan, Ph.D.