Molecular Characterization of the Na^+/H^+ Exchanger in Human Disease

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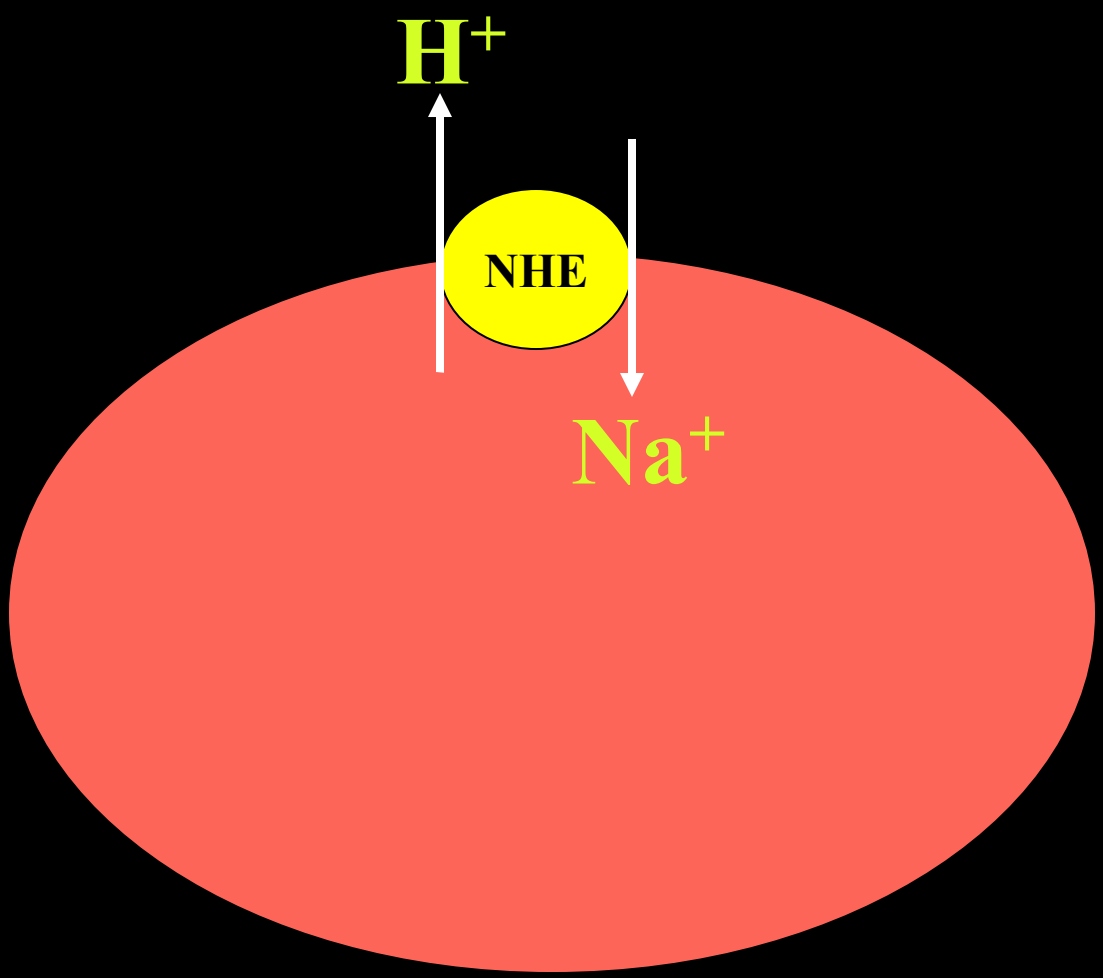
The Na^+/H^+ Exchanger

Plasma Membrane Glycoprotein

1 Intracellular H^+ for 1 Extracellular Na^+

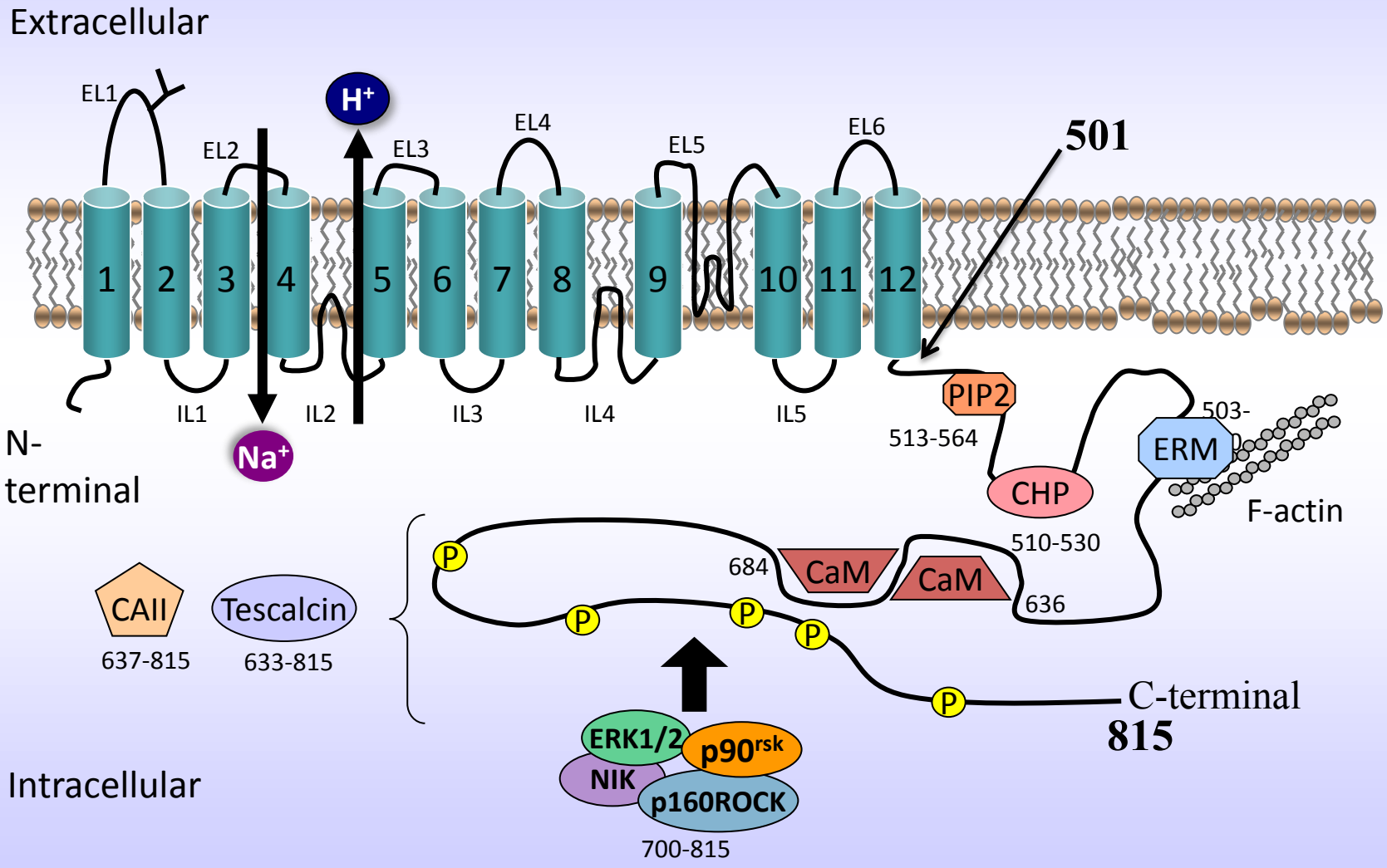
NHE1 ubiquitous,

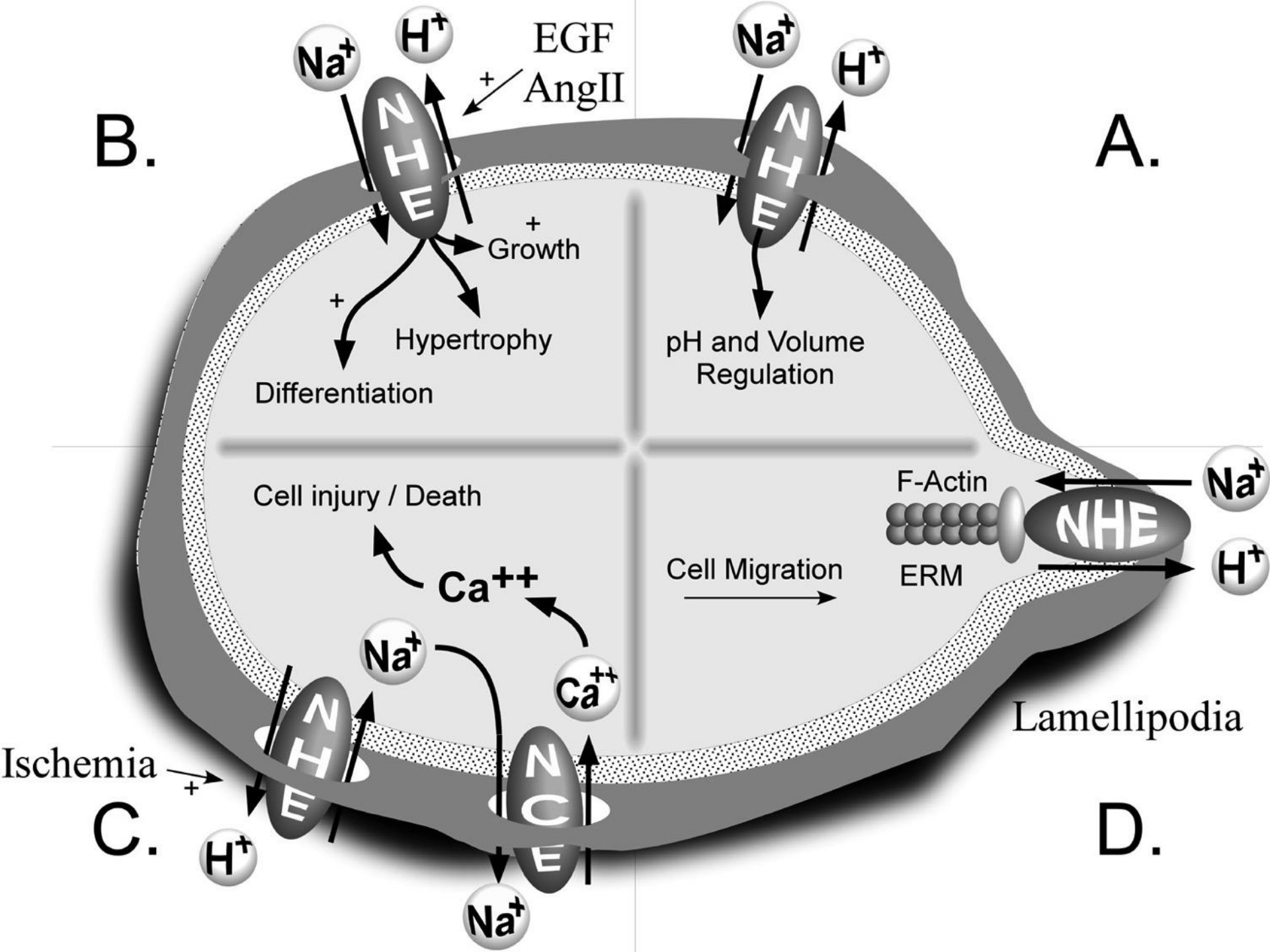
NHE2-10 restricted



Mammals (All tissues)

Model of NHE1




B.
A.
C.
D.
EGF
AngII
Na+
H+
NHE
Growth
Hypertrophy
Differentiation
pH and Volume Regulation
Cell injury / Death
Ca++
F-Actin
ERM
Cell Migration
Lamellipodia
Ischemia
NCE

Characterization of the Na^+/H^+ Exchanger in Two Human Diseases

One common
One rare

Common Disease

Breast Cancer

1 in 9 (or 1 in 8) women

Metastatic Triple negative breast cancer

< 30% survive 5 years

Our Study

Role of

the Na^+/H^+ Exchanger in Breast Cancer

NHE1 and Breast Cancer and Metastasis

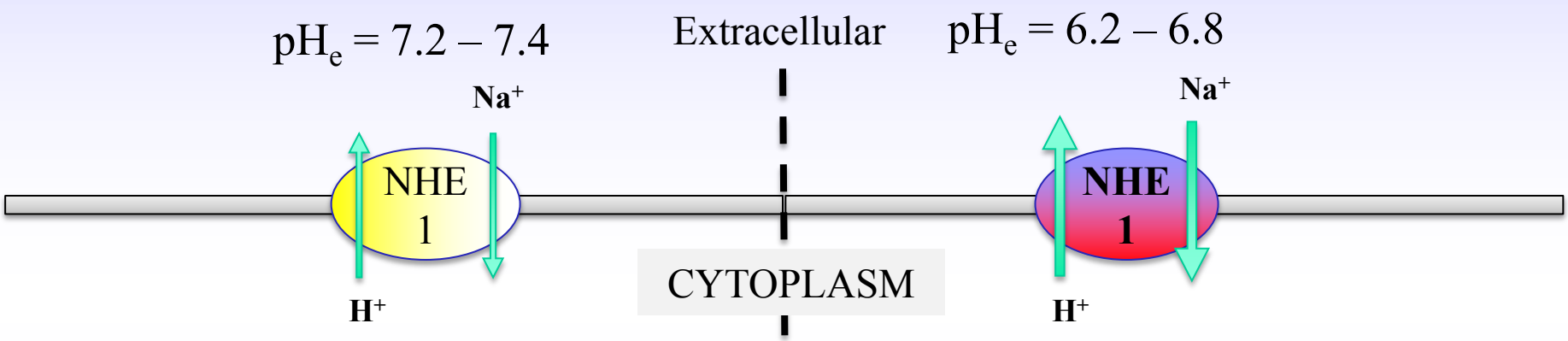
NHE1 is more critical in pH regulation in breast cancer cells

Becomes Hyperactive

Acidifies extracellular microenvironment

Promotes Metastasis

NHE1 in the Tumor Microenvironment



Normal Cell

- Resting pH_i: 6.9 – 7.1
- Proton gradient low; outwards
- NHE1 activated by acidification

Transformed/Tumor Cell

- Resting pH_i: 7.2 – 7.7
- Proton gradient high: Inwards
- Constitutively active NHE1

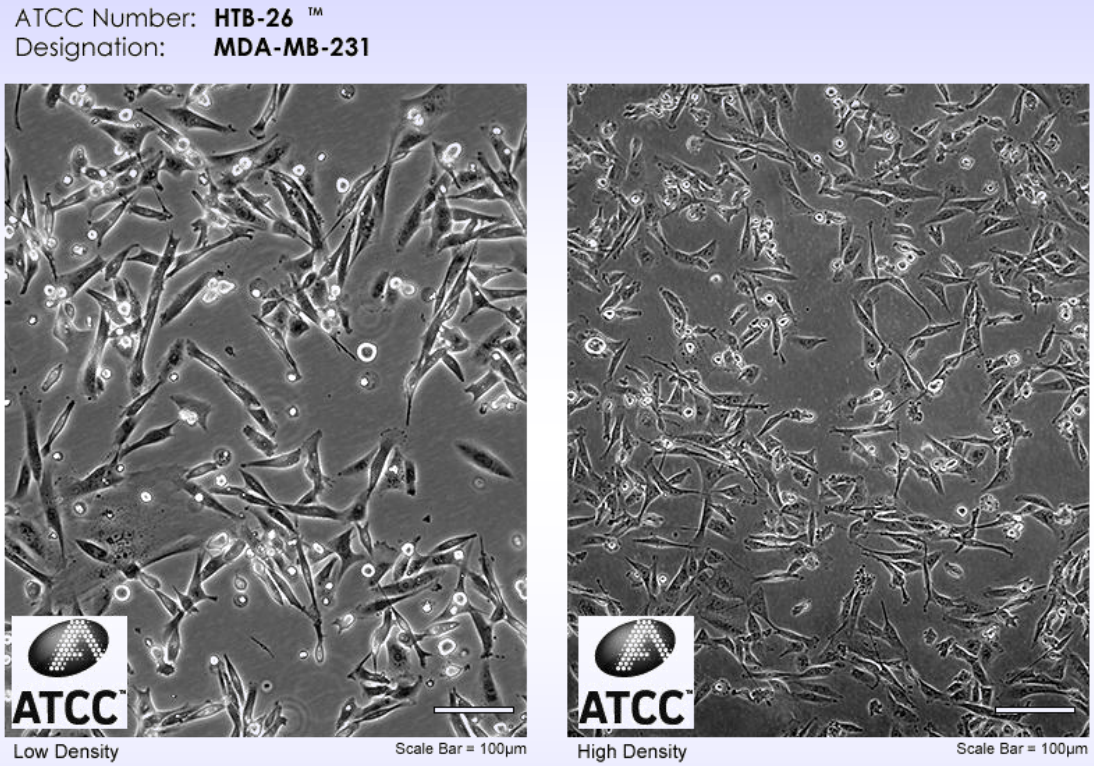
Reshkin and others work

Hypothesis, NHE1 contributes to breast cancer tumor growth and metastasis

Tested the effect of NHE1 knockout and inhibition on various parameters

Cells and Experimental Conditions

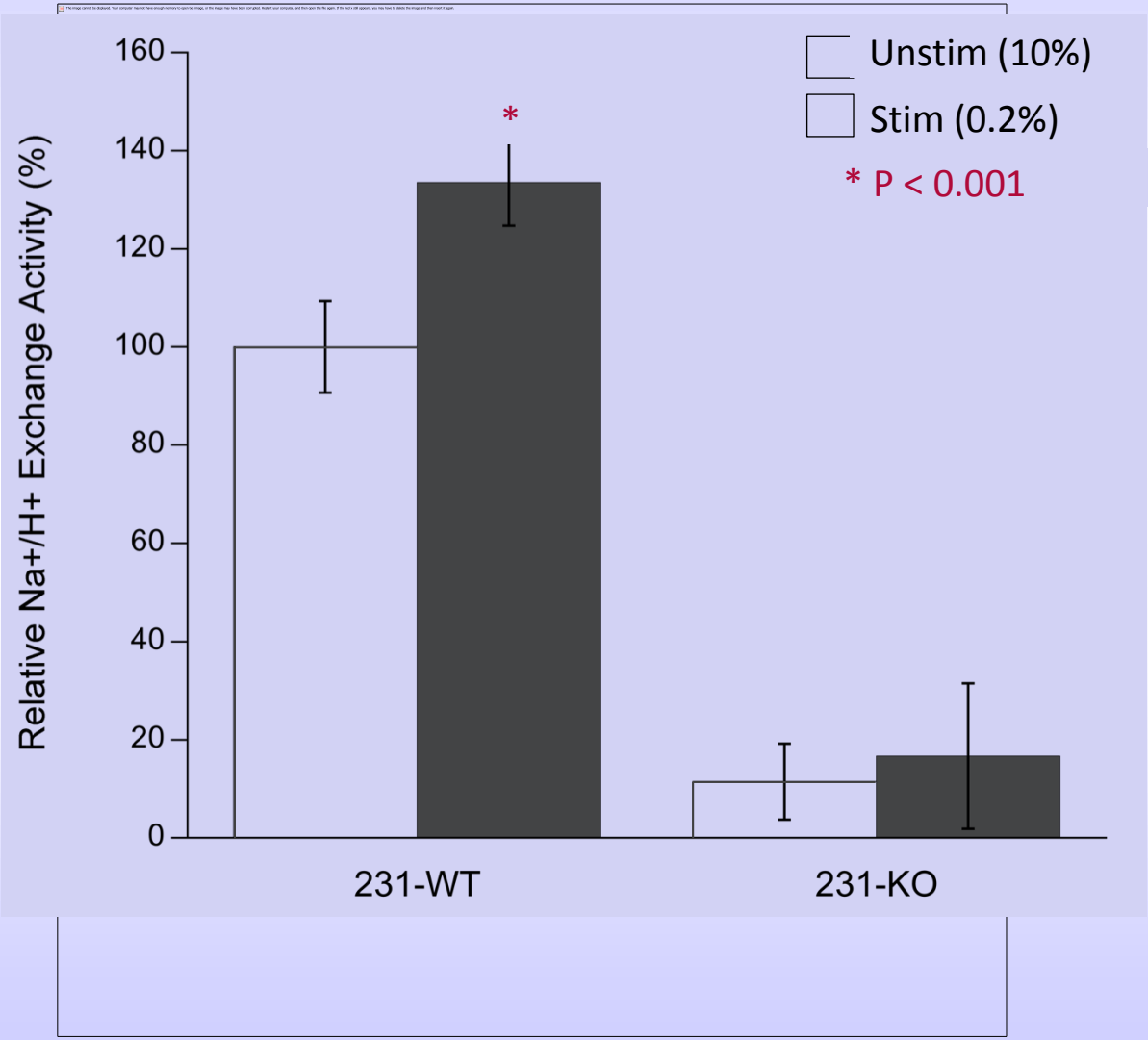
- MDA-MB-231
  - Triple negative
    - Estrogen Receptor
    - Progesterone Receptor
    - Her2/Neu Receptor
  - Highly invasive
  - Endogenous NHE1



- Experimental Culture Conditions
  - Normal serum (10%): Resting (UNSTIM)
  - Low serum (0.2%): Hyperactive NHE1 (STIM)

Generation of 231-KO Cells

WB: Loss of NHE1 expression

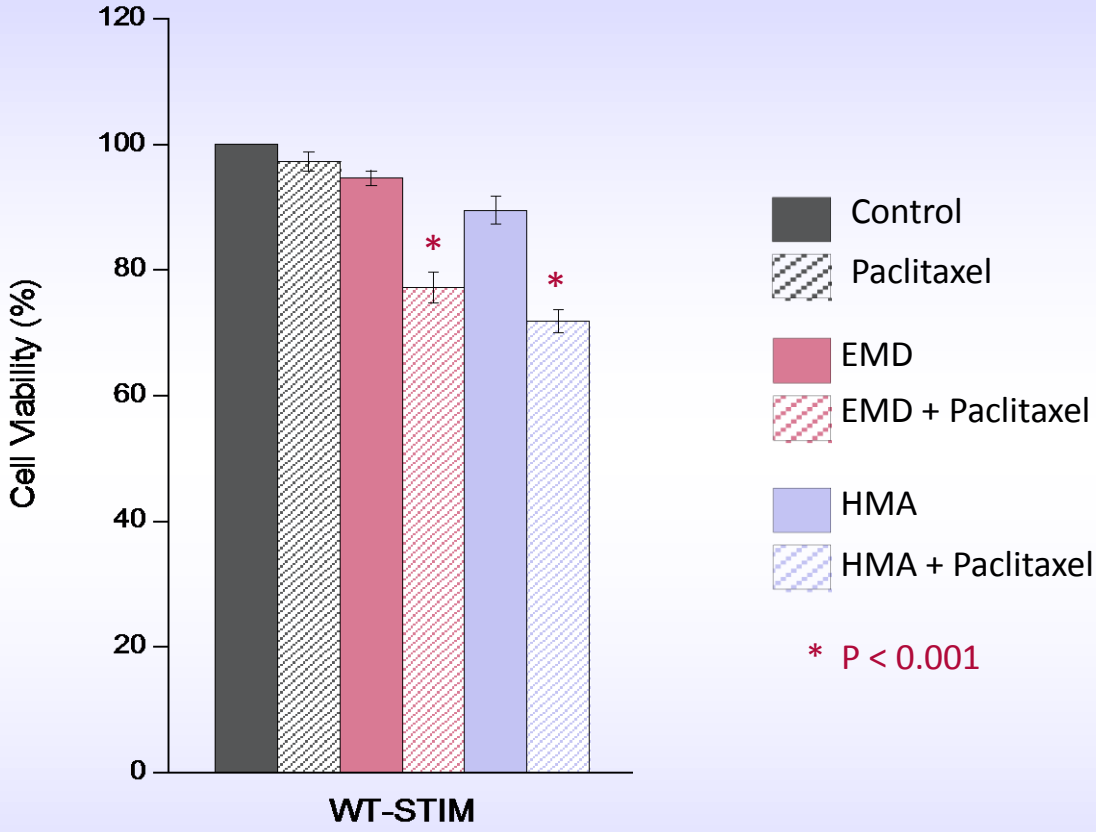


Does loss or inhibition of NHE1 change paclitaxel effects on MDA-MB-231 cells?

Paclitaxel

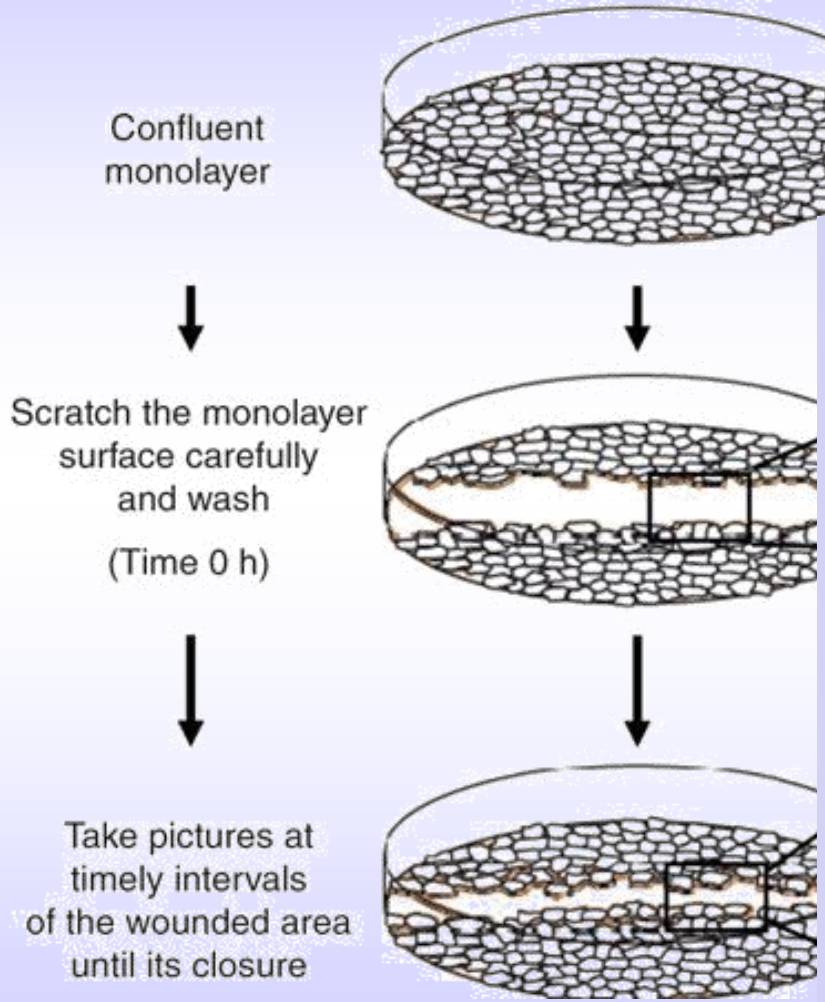
- Chemotherapy drug for breast and ovarian cancer
  - IC_{50} 2.4 nM in MDA-MB-231 cells [Nakayama *et al*, 2009]
  - Used: 1 nM

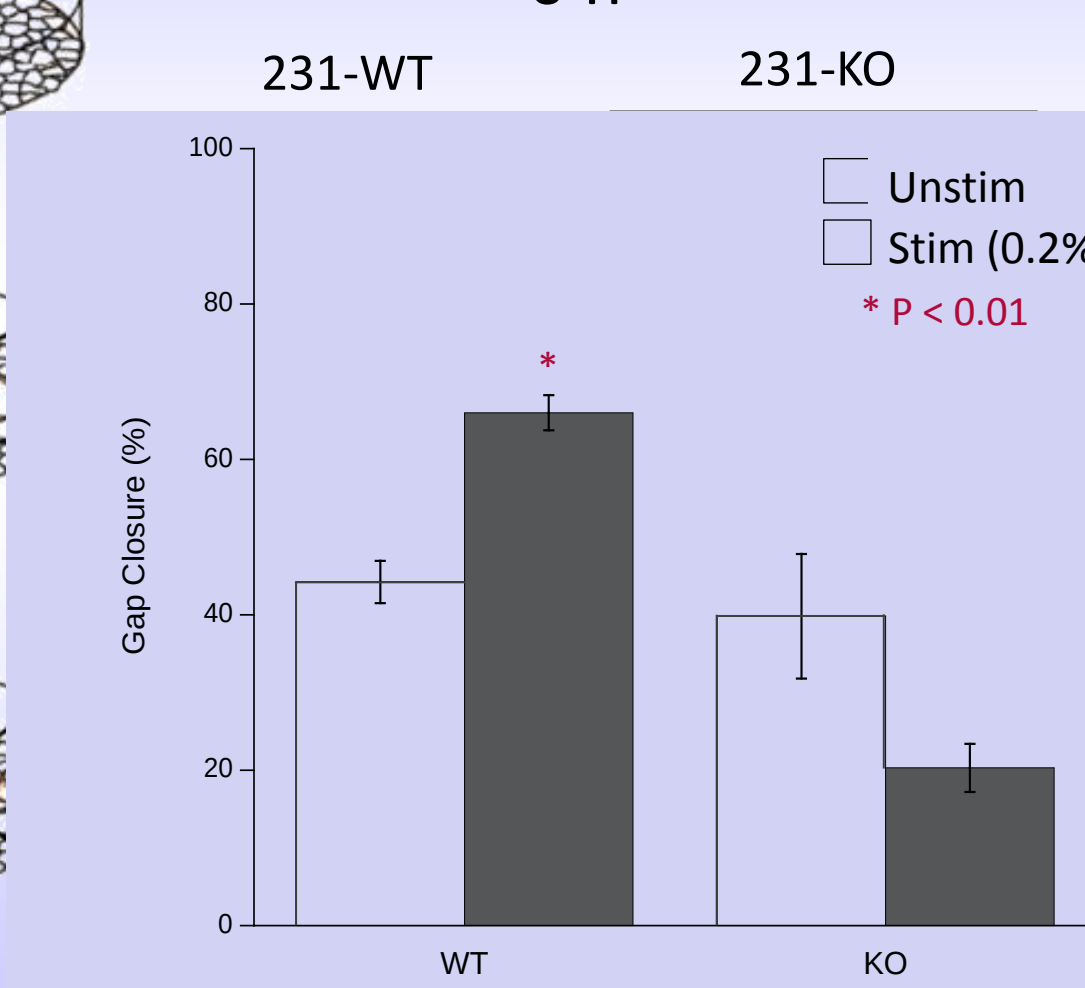
Cell Viability: Effect of Paclitaxel ± EMD or HMA



- NHE1 inhibition increases susceptibility to paclitaxel-mediated cell death

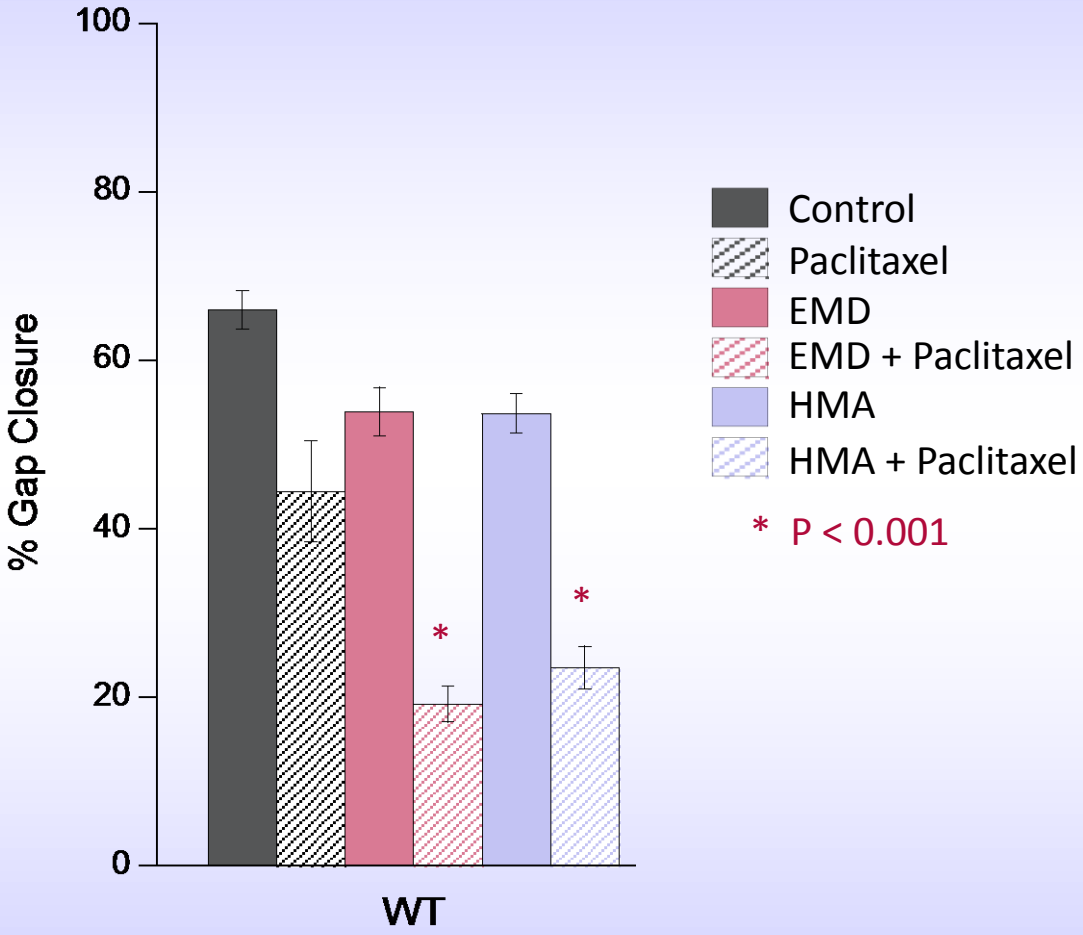
Wound-Healing Assay: Migration
Wild type vs. KO





Adapted from Moreno-Bueno *et al*, 2009 (*Nature Protocols*)

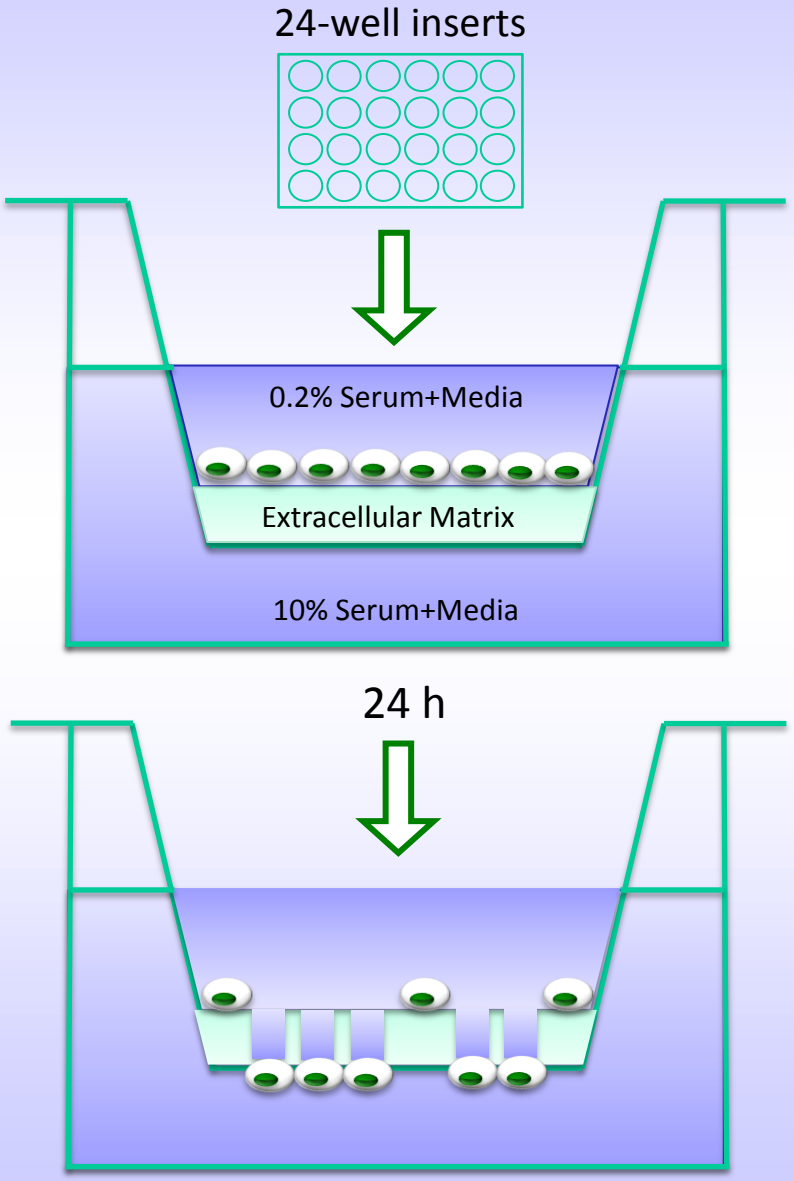
Migration at 18 h: Effect of Paclitaxel ± EMD or HMA



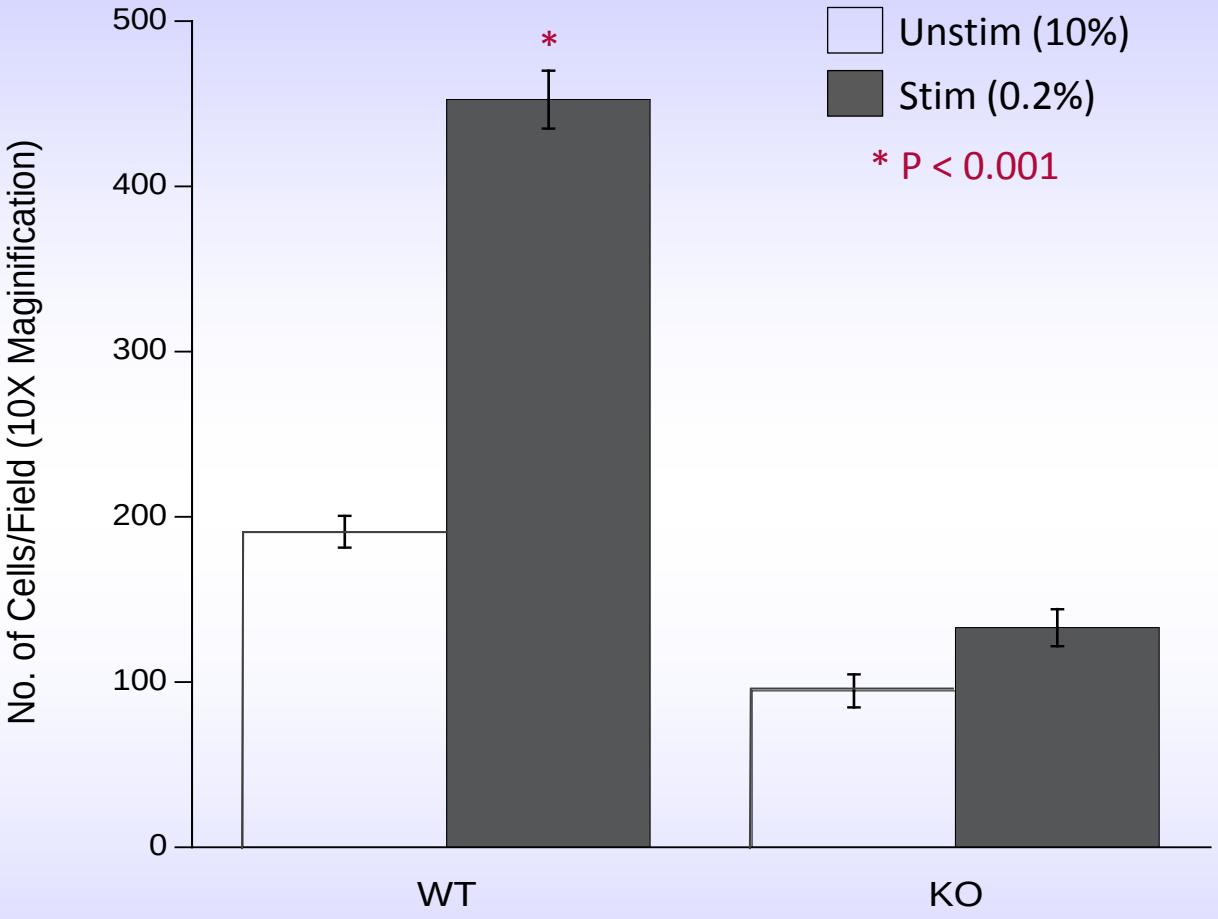
- NHE1 inhibition with EMD or HMA plus paclitaxel greatly decreases the rate of migration of stimulated 231-WT cells

Boyden Chamber Method: Cell Invasion

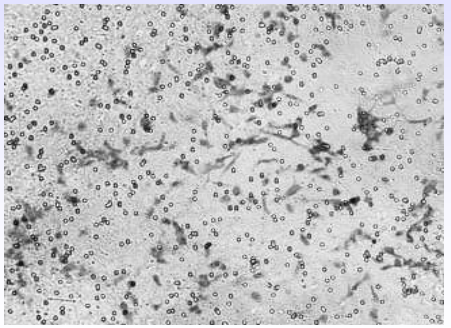
- Invasion Assay:
  - Measure of metastatic potential
  - Invasive cells move through pores in Matrigel-coated



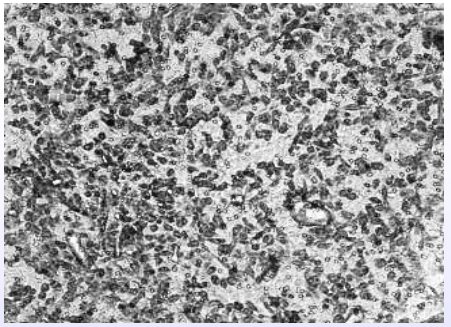
Invasion at 24 h: Effect of NHE1 deletion



KO-STIM

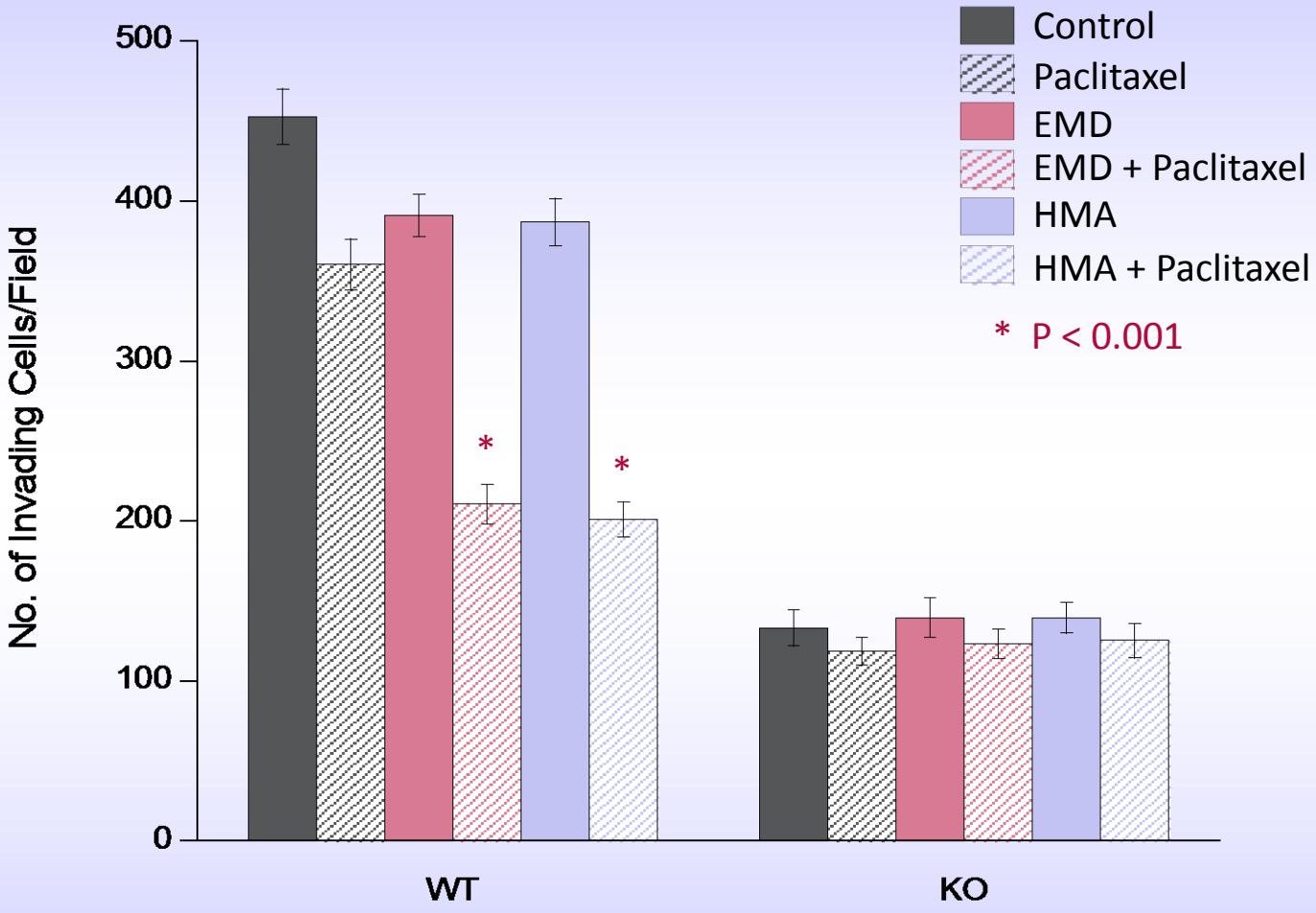


WT-STIM



- Loss of NHE1 greatly reduces the invasive potential of 231-KO cells compared to the wild-type

Invasion: Effect of Paclitaxel ± EMD or HMA



- NHE1 inhibition synergizes paclitaxel responses

Effect of NHE1 Knockout on Xenograft tumor growth in mice

Summary (Part I NHE1 in a common disease)

- Loss of NHE1 decreases tumor growth *in vivo*
- Inhibition of NHE1 in tumor mimetic conditions
  - Increases TNBC susceptibility to Taxol
  - Decreases metastatic potential

NHE1 inhibitors: Potential adjuvant to chemotherapy?

Future Directions

- NHE1 inhibitors + Taxol: Does it work *in vivo*?
- How is NHE1 regulated in TNBCs?

Characterization of the Na^+/H^+ Exchanger in a Rare Human Disease

Mutations in SLC9A1, Encoding NHE1, Cause Ataxia–Deafness Lichtenstein–Knorr Syndrome

Collaboration, Claire Guissart, Michel Koenig, Montpellier, France

Xiuju Li

((2015) Human Molecular Genetics 24(2):463-70.)

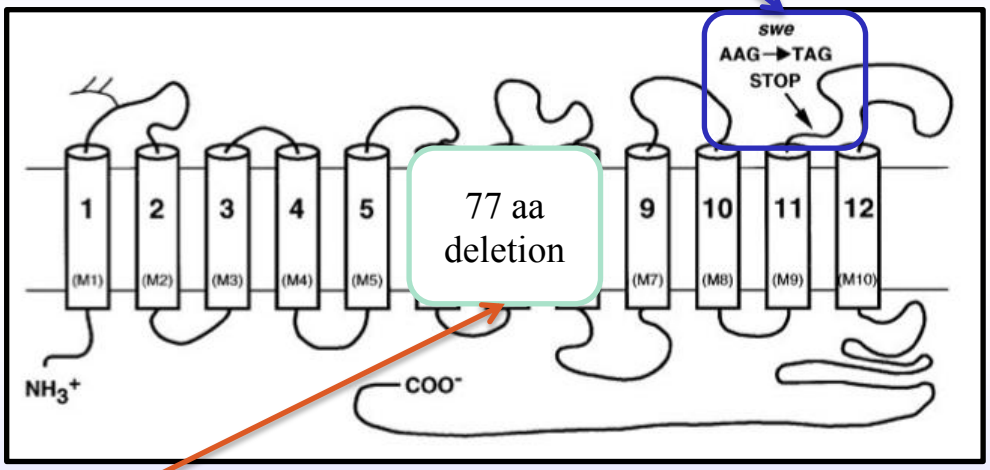
Mouse NHE1 Knockout Models

Cell, Vol. 91, 139–148, October 3, 1997, Copyright ©1997 by Cell Press

Sodium/Hydrogen Exchanger Gene Defect in Slow-Wave Epilepsy Mutant Mice

Cox et al.

Spontaneous mouse mutant p.Lys442*



Bell et al.

Am J Physiol. 1999 Apr;276(4 Pt 1):C788-95.

Targeted disruption of the murine Nhe1 locus induces ataxia, growth retardation, and seizures.

Lichtenstein–Knorr Syndrome

-autosomal recessive
-1930 by H. Lichtenstein and A. Knorr
-sensorineural hearing loss and cerebellar ataxia
-cause unknown

Identified

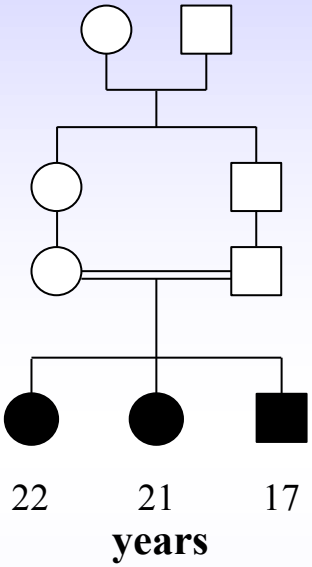
Consanguineous family

-recessive cerebellar ataxia/deafness
-exome sequencing
-bioinformatics ranking of genetic variants

CLINICAL Summary

Lichtenstein-Knorr syndrome

Ataxia
Deafness



- Turkish origin, 3 affected
- 1st degree consanguinity
- Cerebellar and posterior column ataxia
- delayed walking at ages ranging from 18 months to 5 years
- Deafness (profound in the sisters, moderate in the younger brother)
- Retarded language in the oldest sister
- Growth retardation and microcephaly (brother only)

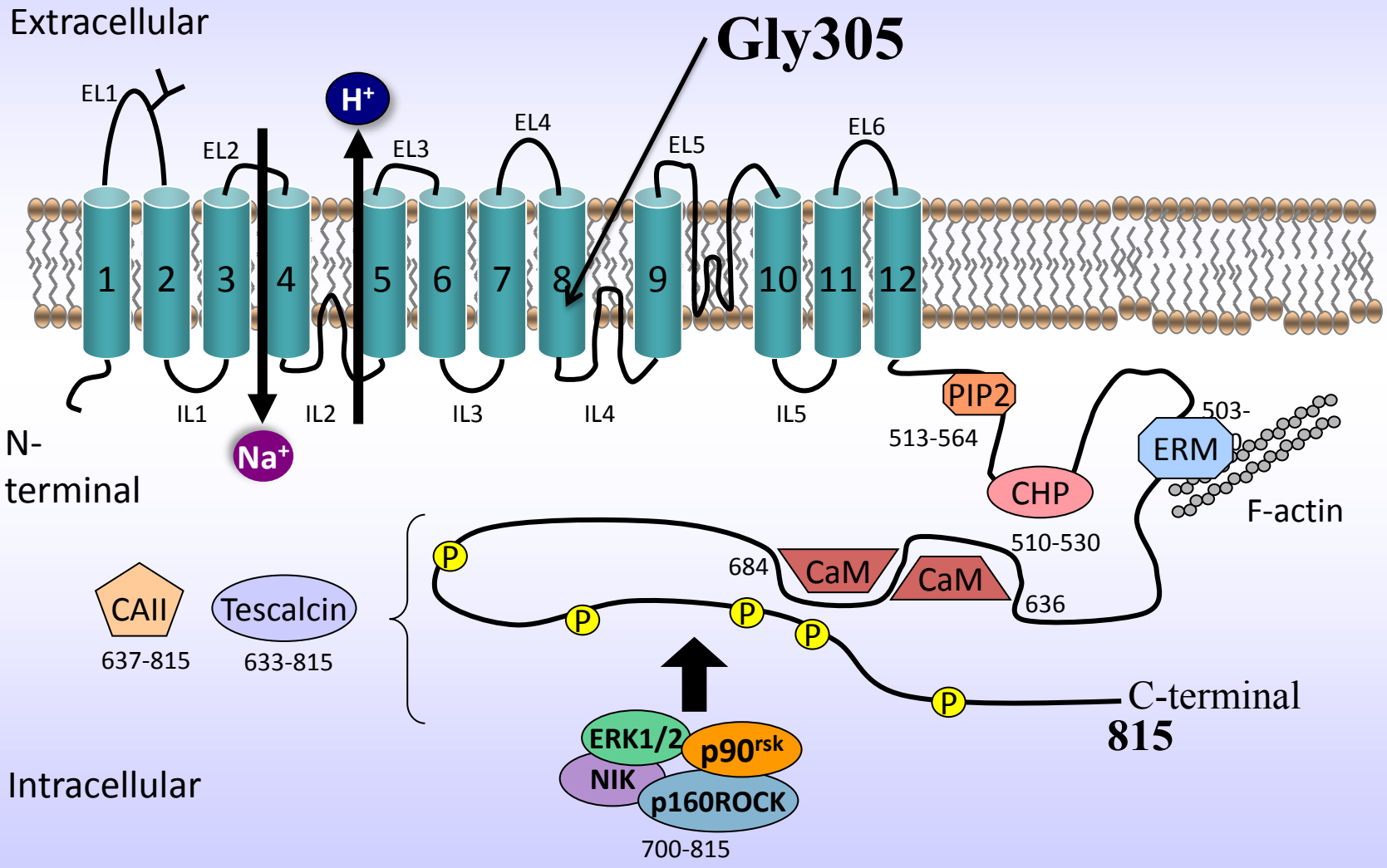
Localization of the defect to the NHE1 (SLC9A1) Gene

SNP (single nucleotide polymorphism) Mapping

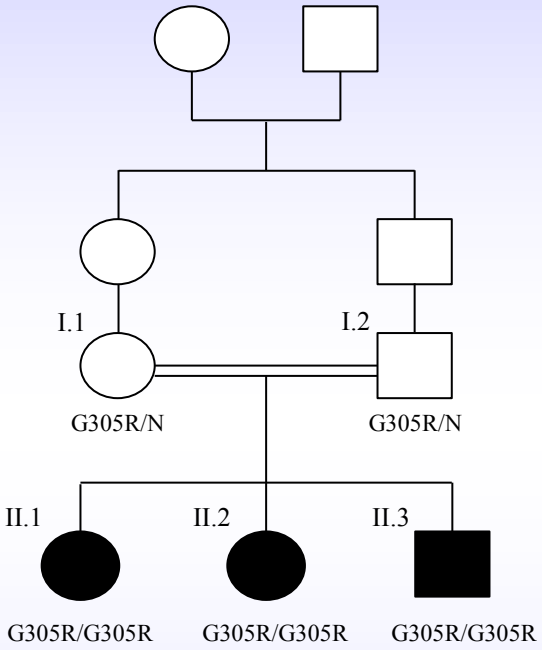
Exon (Exome) Sequencing

Glycine 305 of NHE1 Gly 305 Arg

Putative Location of Gly305

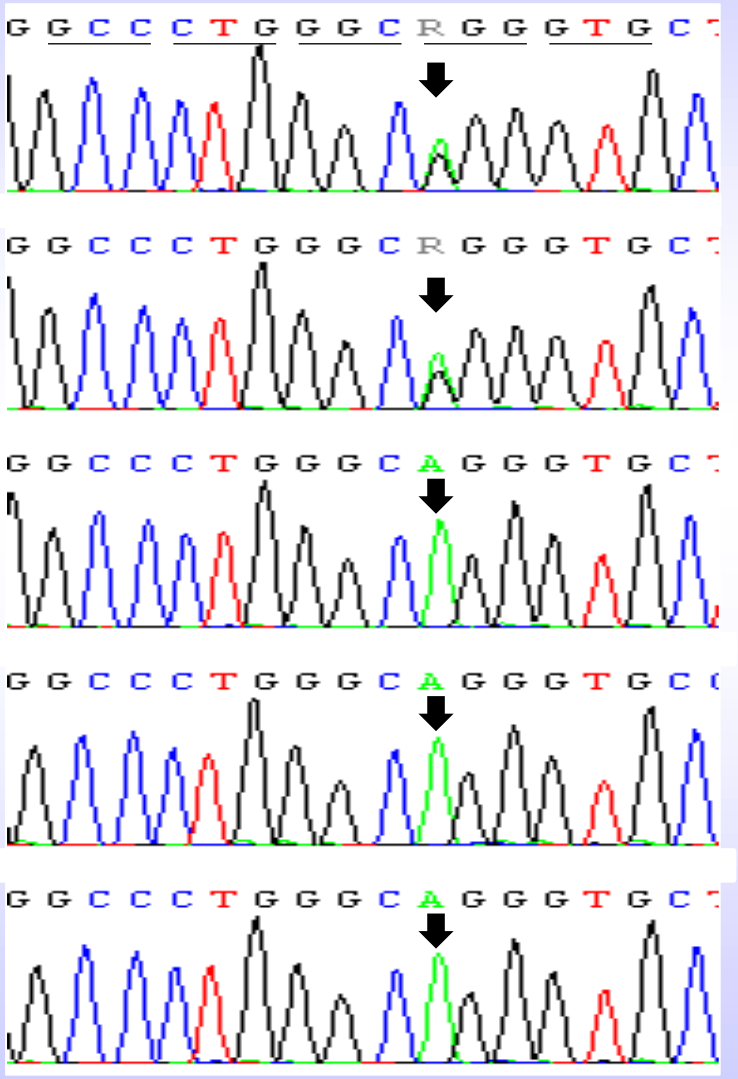


Inheritance, One bp Change, Gly305Arg



Predicted deleterious by SIFT (score=0.02)

Gly GGG
Arg AGG



Conservation of Gly305 in Na/H Exchangers

p.Gly305Arg

```
Homo sapiens NHE1          EFANY--EHVGIVDIFLGFLSFFVVALGGVLVGVVYGVIAAFTSRFTS
Homo sapiens NHE2          SFCQM--KTIETIDVFAGIANFFVVGIGGVLIGIFLGFIAAFTTRFTH
Homo sapiens NHE3          SFVALGGDNVTGVDCVKGIVSFFVVSLGGTLVGVVFAFLLSLVTRFTK

Gallus gallus              EFANF--EQVTIIDMVLGFLSFFVASLGGVFVGVIYGLIAAFTSRFTS
Alligator sinensis         EFANI--KQVTIIHILLGFISFFVVSLGGVFIGIIYGIVAAFTSRFTS
Xenopus tropicalis         EFAAL--EQITFRDISLGFLSFLVVALGGVFVGLVYGIIAAFTSRFTS
Lepisosteus oculatus       EYAGV--GSVTFLDVFLGVVCFLVVALGGIFVGAVYGILAAFTSRFTS
Bombyx mori                AYTEMGPSRLVYTDILAGLASFLVVAVGGTCIGVVWGFATGLVTRFTN
Pediculus humanus corporis AYNEMGPSNILYTDVLSGLASFLVVALGGTIIGIVWGFLTGLVTRYTD
Necator americanus         EFKEL--DSIGFLDCFMGFLAFLCVSLGGLAIGLFFGFMSAFVTKFTK
                           THKEILLGFANFLVVSLGGTLMGVLWGFFTAFVTKYTD
                           EGGMVALGIFSMFVVSIGGIVIGLLYGMLAAFFTKYTP
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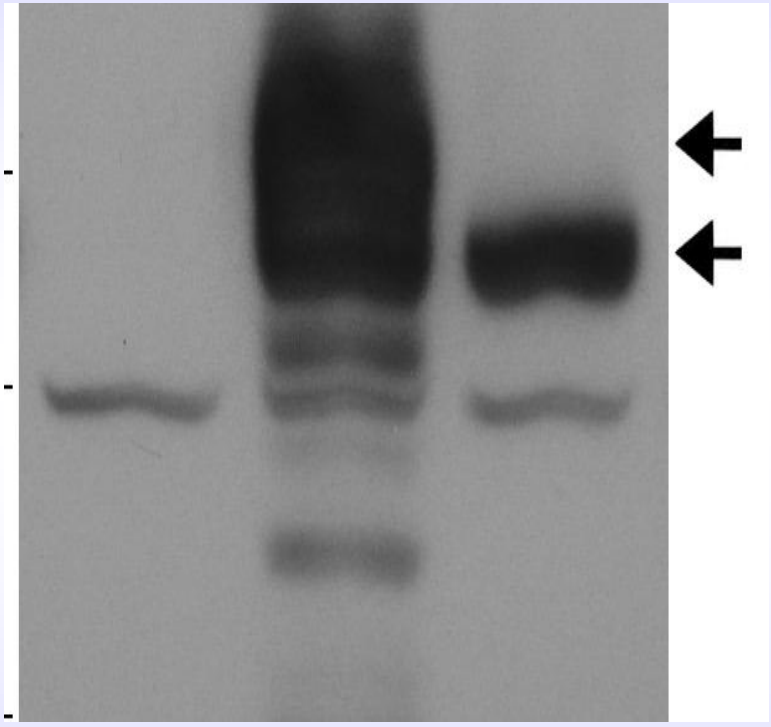
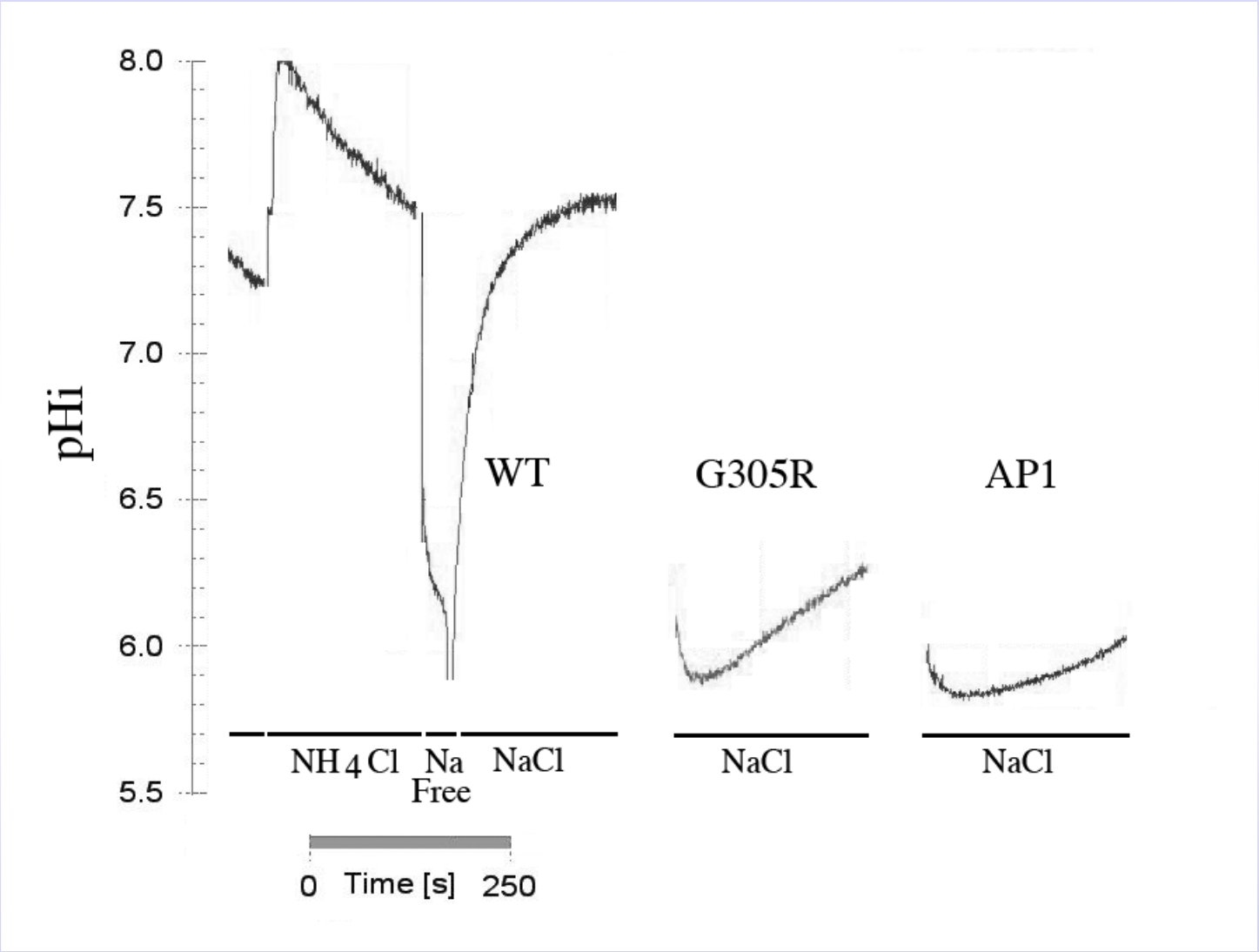
transmembrane

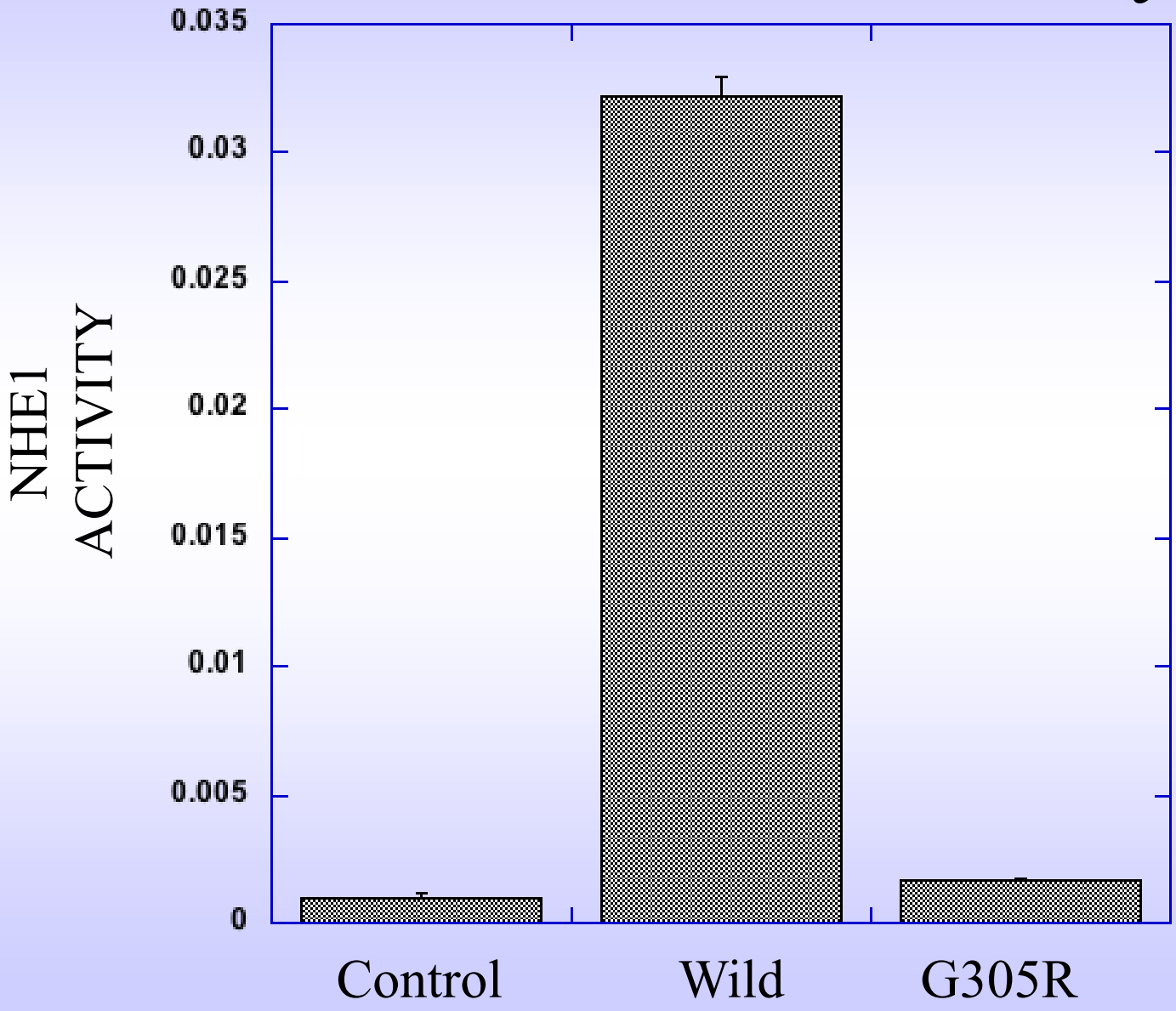
Western blot of Wild Type and Mutant NHE1 Expressed in AP-1 Cells



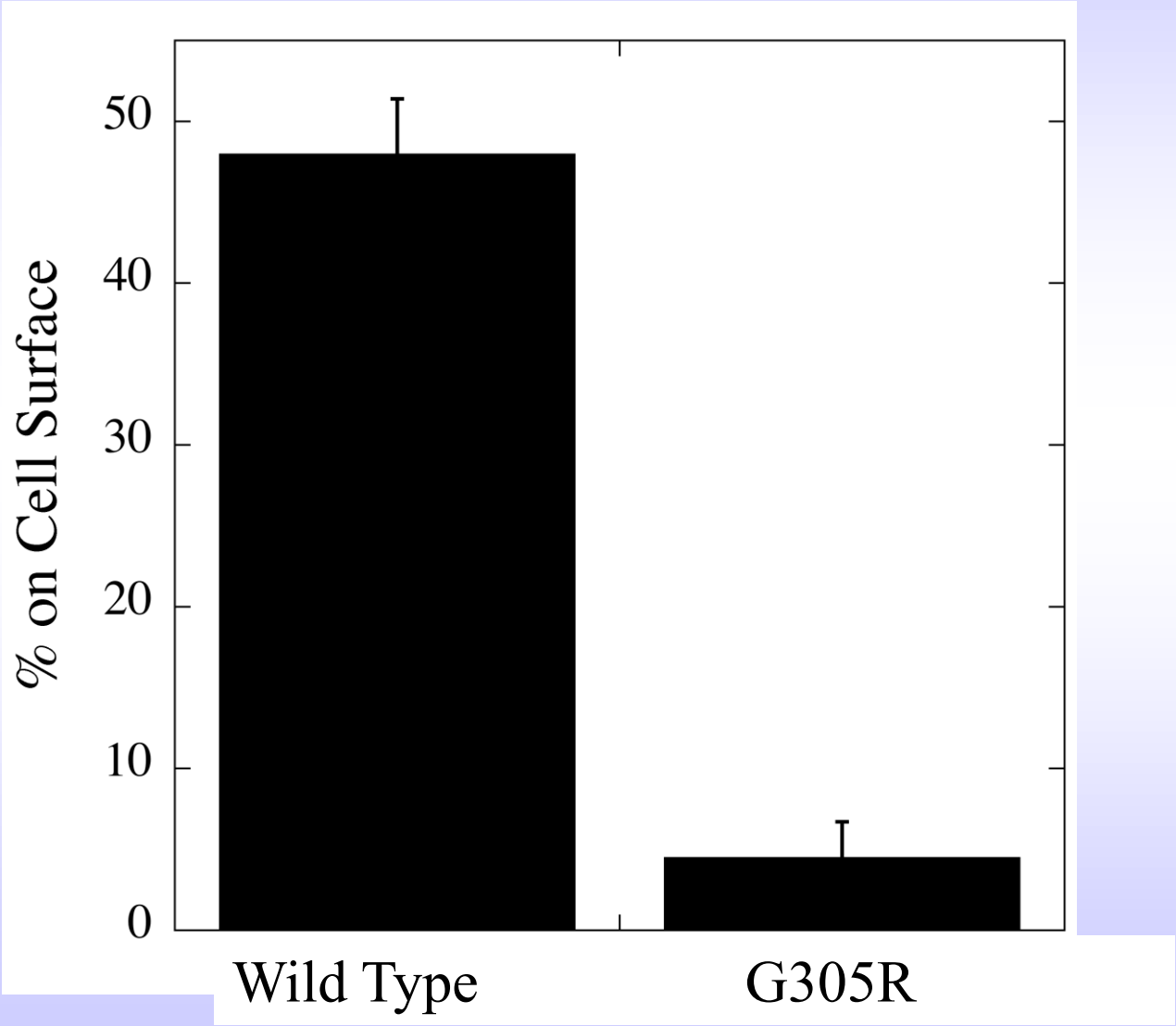
NHE1 Activity



Mutant G305R Activity

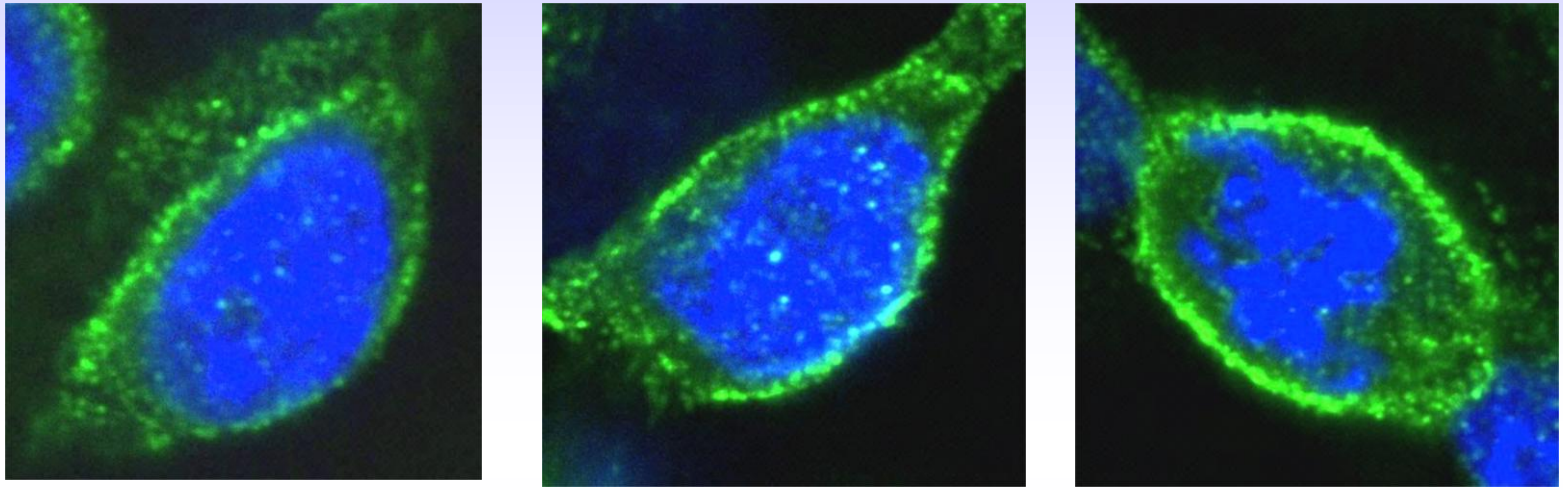


Cell Surface Targeting is defective

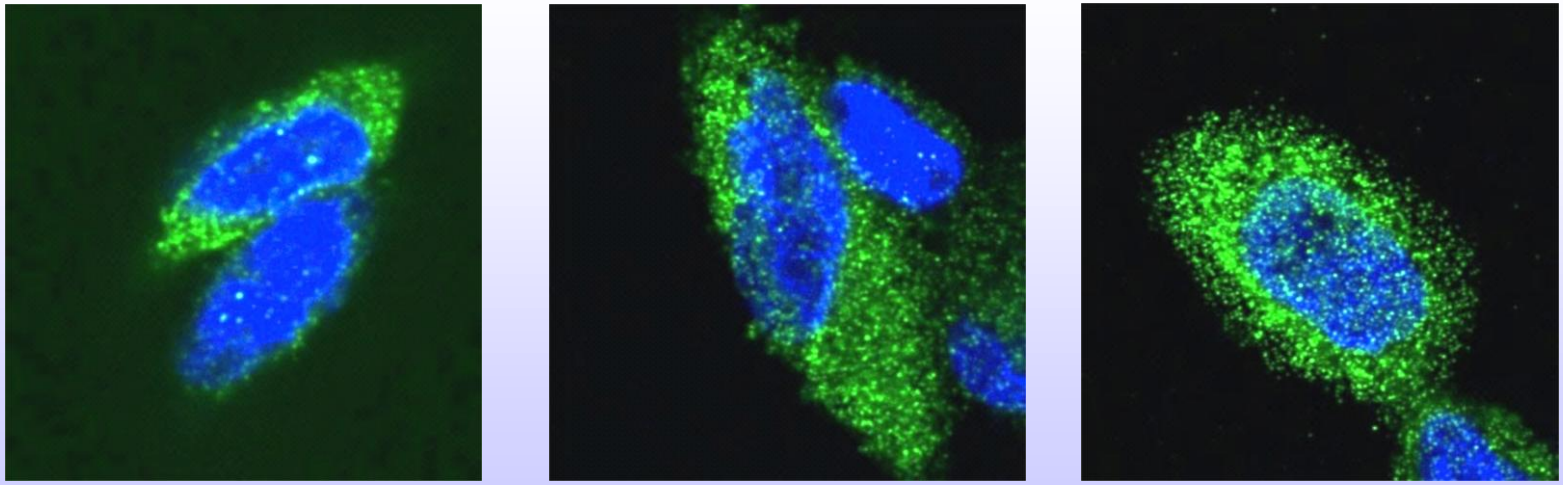


Immunocytochemistry

WT-NHE1



G305R



Summary

-The disease Lichtenstein–Knorr Syndrome is due to a defect in NHE1 gene (SLC9A1)

-Gly305Arg mutation, results in defective activity and targeting

Experiments are underway to correct the defect

Acknowledgements

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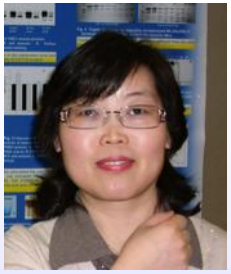

CIHR IRSC

Supported by the CIHR, CBCF

Cell Surface Targeting is defective