Targeting voltage-gated K+ channels in cancer reveal new biochemical pathways and therapeutic opportunities

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Voltage-Gated Ion Channels in Cancer Cell Proliferation
Vidhya R. Rao, Mathew Perez-Neut, Simon Kaja and Saverio Gentile

Cancers 2015, 7(2), 849-875
Potassium channels have been found overexpressed in a variety of tumors of different histogenesis but absent in healthy cells from which the respective tumor are derived.

- Rhabdomyosarcoma
- Leukemia
- Melanoma
- Neuroblastoma
- Breast cancer
- Pancreatic cancer
- Intestine

hERG1 channel expression pattern in tumors
Breast cancer facts sheet

- 1 in 8 women will be diagnosed with breast cancer in their lifetime.
- 1 in 33 women will die from breast cancer.
- Men have the possibility of developing breast cancer as well.

- hERG1 expression is associated with higher mortality
- hERG1 is more abundantly expressed in ER-neg

Overall Survival Kaplan-Meier Estimate
www.cbioportal.org/public-portal
Previous investigation have shown that blocking hERG1 current activity leads to cancer cell death. However, because of the deleterious effects of hERG1 channel blockers on heart performance these drugs cannot be used in cancer therapy.

Recently, a group of structurally different hERG1 activators have been discovered. It has been shown that mammalian hearts can tolerate these drugs. They exert less dramatic side effects compared to hERG1 channel blockers.

The therapy was successful but the patient died.

Can we manipulate hERG1 to fight breast cancer?
Q1 - What are the consequences of hERG1 potassium channel stimulation in breast cancer cells?

Q2 - What is the biochemical signaling activated by stimulation of hERG1 potassium channels in breast cancer cells?
A&D) hERG1 channel expressed in ER-neg breast cancer cell line SKBr3 and CHO cells.
B&D) Effects of the NS1643 (50μM) on hERG1 currents.
C&F) Effects of NS1643 on proliferation rate of hERG1 negative cells.
Stimulation of hERG1 channel causes cell cycle arrest in G\textsubscript{0}/G\textsubscript{1}

A, B & D) Cell cycle phase distribution of SKBr3 before and after NS1643 or NS1643+E4031 (herg1 blocker) treatment.


Lansu et al.
Cell death and Disease 2013
Cyclin E2
- Breast cancer marker
- Predictor for poor prognosis
- Over-expressed in recurrent tumors

P21cip/Waf
- Loss of function in cancer
- Predictor for poor prognosis

Cyclin E2
actin

Contr 50μM
48 kD

Contr 50μM
20 kD
42 kD
E-type cyclins

**cyclin E1**

- **NLS**
- **P**
- **CLP**
- **CLS**

**cyclin E2**

- **NLS**
- **P**
- **CLP**
- **CLS**

---

**P24864 CCNE1_HUMAN 1**

MPRRERRERDAKERTM----KEDGGAESRASRKRKANKTVFLQDPDEEMAKIDRTARDQ 56

**O96020 CCNE2_HUMAN 1**

MSRSSRLQAKQQPQPSQTESPQAEQIIAQAKKRKTQ----DVKKRREEVTKKHQYEIRN 56

---

**P24864 CCNE1_HUMAN 57**

CGSQPWDN-NAVCADPSCSIPTDPKEDDDRVYNPSTCKPRIIAPRSGASLPVLSVANREE 115

**O96020 CCNE2_HUMAN 57**

C----WPPVLSSGGISPQIIETPHKEIGHTSDSRFTNYRKNLFINPSPDLDSWGCSE 112

---

**P24864 CCNE1_HUMAN 116**

VWKIMLNKEKTYLRDQHFLEQHPLLQPKMRAILLDLWMEVCEVYKHLRETIFYLAQDDFR 175

**O96020 CCNE2_HUMAN 113**

VWNMLKESRYVHDKEFLSDLEQMRSLDLLWLECVYTLHRETIFYLAQDDFR 172

---

**P24864 CCNE1_HUMAN 176**

YMATQENVKTLQLIGISLFIASKLEEIYPPKLQEFAYVTDGACSGDEILTMELMIMK 235

**O96020 CCNE2_HUMAN 173**

FMLQDKINKMLQIGITSFLIAKLEEIYAPKLQEFAYVTDGACSEIDLRMLIELIIK 232

---

**P24864 CCNE1_HUMAN 236**

ALKWRLSPLTIVSWLNVYMQVAYLNDLHEVLLPQYPQIQIFIAEILLDLVCVDCLDFP 295

**O96020 CCNE2_HUMAN 233**

ALKWELCPVITISWLNLQVDAKDAVLPQYQSSEQTFIQIAEILLDLCLAIDESLDFQ 292

---

**P24864 CCNE1_HUMAN 296**

YGILAASLEYHFSSELQMKVSGYQWDIENCVKWMVPFAMVIRETSGSLKHLFQAVDE 355

**O96020 CCNE2_HUMAN 293**

YRILTAALCHFTSIEVVKASGLEWSIDSECVDWMVPVFVNVKSTPSVKLTKFPPPME 352

---

**P24864 CCNE1_HUMAN 356**

DAHNIQTHRSDLDDLKARAKAMLSQNRRASLPSGLLTPPSGKQSSGPEMA 410

**O96020 CCNE2_HUMAN 353**

DRHNIQTTHYNLAMLEEVNINTFRKGGQLSPVNGGIMTPKSTEKPGKH--- 404

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hERG1 stimulation selectively targets cyclin E2 for degradation

(2 hours)

**A)**

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**B)**

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**C)**

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Mathew Perez-Neut
Perez et al.
Oncotarget Jan 2015
hERG1 stimulation selectively targets cyclin E2 for degradation
Stimulation of hERG1 leads to $\text{Ca}^{2+}$-dependent cyclin E2 degradation.
Perez et al. “Stimulation of hERG1 channel activity promotes a calcium-dependent degradation of cyclin E2, but not cyclin E1, in breast cancer cells” Jan. 2015 - Oncotarget
Cyclin E1

Cyclin E2

Two green apples are shown, labeled as Cyclin E1 and Cyclin E2.
- A p53 loss-of-function mutation is the most frequent mutation leading to cancer.
- p21 acts as a master effector of multiple tumor suppressor pathways.
- p21 expression is tightly controlled by the tumor suppressor p53.
Stimulation of hERG1 leads to an increase of the tumor suppressor p21.

Erα/PR/HER2/p53

- Erα: Estrogen Receptor alpha
- PR: Progesterone Receptor
- HER2: EGFR2
- p53: → p21

Perez et al. Oncotarget Apr 2015
Stimulation of hERG1 activates transcription of the tumor suppressor p21

Effect NS1643 on NFAT-dependent transcription (at 8hr)

Effect NS1643 on p21-transcription (at 8hr)

Effect NS1643 on p21 protein expression (at 24hr)

Time course (Hr) of the effect NS1643 on NFAT-dependent transcription in SKBr3 cells.

Effect NS1643 on NFAT-reporter activity

Luciferase activity
NFAT activator

Calcineurin

CaM

TRPV6 (?)

Ca\(^{2+}\)

Cyclin E2

Cyclin E1

Proliferation

p21

Trancriptor

CaM

K\(^{+}\)

Ca\(^{2+}\)
Thanks to:

Katherine Lansu
(2011-2013)
now Ph.D. candidate UNC

Mathew (one t) Perez
Psychedelic Tech 2013-present
now Ph.D. candidate U of C.

Kadhya Rao
(Psychodoc 2014-present)

Richard Miller
(Northwestern Univ.)

Clodia Osipo
Loyola Cancer Center
...it is not just hERG1

Celecoxib: A Specific COX-2 Inhibitor With Anticancer Properties

Differential Effects of Selective Cyclooxygenase-2 Inhibitors on Vascular Smooth Muscle Ion Channels May Account for Differences in Cardiovascular and Anti-Cancer Activity

Lioubkov I. Brueggemann, Alexander R. H. K. M. Byron

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<th>CHO</th>
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</table>

- NS1643
- PD118057
- Dymethylcelecoxib
- Celebrex®
Perez et al. “hERG1Kv11.1 activation stimulates transcription of p21wafcip in breast cancer cells via a calcineurin-dependent mechanism” Apr. 2015 - Oncotarget
- Ion channels are pore-forming membrane proteins whose functions include establishing electrical signals by gating the flow of ions across the cell membrane.

- Ion channels are key components in a wide variety of biological processes that involve both rapid and slow changes in cells.

**milliseconds**

- Neuronal transmission (270 miles/hr)
- Muscle contraction

**minutes**

- Cell volume
- T-cell activation
- Transport of nutrients
- Secretion

**hours**

- Proliferation

Cancer
Perez et al. “hERG1Kv11.1 activation stimulates transcription of p21wafcip in breast cancer cells via a calcineurin-dependent mechanism” Apr. 2015 - Oncotarget
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NS1643 promotes NFAT nuclear translocation
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