Understanding Magnesium and Magnesium Transporters in Cancer: .. How Far? ... How Close?

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The Hallmarks of Cancer

Hanahan and Weinberg, Cell, 2000
Hanahan and Weinberg, Cell, 2011

Emerging Hallmarks

Deregulating cellular energetics

Avoiding immune destruction

Genome instability and mutation

Tumor-promoting Inflammation

Enabling Characteristics
Carcinogenesis:

- Oxidative stress
- DNA damage repair

Tumour growth and spreading:

- Proliferation, Metabolism
- Angiogenesis
- Invasion
- Metastasis

Tumour treatment:

- Neprotoxic agents
- Therapeutical outcome?
Magnesium in Carcinogenesis

**In Vitro mechanisms**

Oxidative stress $\uparrow$ < Mg$^{2+}$

DNA damage repair $\downarrow$ < Mg$^{2+}$
1139 cases and 1210 matched healthy controls with data on both diet and DNA repair capacity (DRC), measured using the host cell reactivation assay to assess repair in lymphocyte cultures. Low dietary Mg intake was associated with poorer DRC and increased risk of lung cancer. The effects were more pronounced among older subjects (>60 years), current or heavier smokers, drinkers, those with a family history of cancer.
Magnesium in carcinogenesis

Epidemiological data

Blood Magnesium, and the Interaction with Calcium, on the Risk of High-Grade Prostate Cancer

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¹ Vanderbilt Epidemiology Center, Vanderbilt-Ingram Cancer Center, Vanderbilt University Medical Center, Vanderbilt University School of Medicine, Nashville, Tennessee, United States of America, ² Department of Urologic Surgery, Vanderbilt University Medical Center, Nashville, Tennessee, United States of America, ³ Urology Associates, Nashville, Tennessee, United States of America

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Magnesium intake and colorectal tumor risk: a case-control study and meta-analysis¹·²·³·⁴

Petra A. Wark, Rosa Lau, Teresa Norat, and Ellen Kampman
Carcinogenesis: in vivo data

Organomagnesium suppresses inflammation-associated colon carcinogenesis in male Crj: CD-1 mice

Toshiya Kuno, Yuichiro Hatano, Hiroyuki Tomita, Akira Hara, Yashinouhiro Hirose, Akihiro Hirata

There has been a marked increase in the understanding of cell and molecular mechanisms underlying a variety of carcinogenic pro-

Azoxymetane
Dextran
Sulphate
- Inflammation score
- Mitotic index (AI)
- Inflammatory cytokines: 
  - TNFα, IL-1β, IL-6, INF-γ,
- MCM2-positive index
- Anaphase Bridging Index (ABI)
- iNOS, Cox-2
Conclusion

• Organo-Mg inhibits inflammation-related mouse colon carcinogenesis by modulating
  – the proliferative activities
  – chromosomal instability of CRC and
  – suppressing colonic inflammation

• Results may suggest potential use of
• organo-Mg for clinical chemoprevention trials of CRC in the inflamed colon.
  • Toshiya K, Carcinogenesis, 2013
Tumour growth and spreading:

- $< \text{Mg}^{2+}$
- Proliferation
- Metabolism
- Angiogenesis

Primary tumour growth

- $< \text{Mg}^{2+}$
- Invasion
- Metastasis
- $< \text{Mg}^{2+}$
- Inflammation

Metastatization
Magnesium and cell cycle regulation

Magnesium Depletion Causes Growth Inhibition, Reduced Expression of Cyclin D1, and Increased Expression of P27KIP1 in Normal But Not in Transformed Mammary Epithelial Cells

Alessandro Sgambato, Federica I. Wolf, Beatrice Faraglia, and Achilles Cittadini

ARTICLE


Magnesium Deficiency Suppresses Cell Cycle Progression Mediated by Increase in Transcriptional Activity of p21Cip1 and p27Kip1 in Renal Epithelial NRK–52E Cells

Akira Ikari,1* Hayato Sawada,1 Ayumi Sanada,1 Chie Tonegawa,1 Yasuhiro Yamazaki,1 and Junko Sugatani1,2

1Department of Pharmaco-Biochemistry, School of Pharmaceutical Sciences, University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422–8526, Japan
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Magnesium and angiogenesis
In vitro data

Influence of extracellular magnesium on capillary endothelial cell proliferation and migration.
S Banai, L Haggroth, S E Epstein and W Casscells

J. Maier, J. Maier, J. Maier, J. Maier
Magnesium and Tumour growth: in vivo data

TRANSPLANTED TUMOR CELLS

Mg 1000 mg/kg

Mg 30 mg/kg

NATO collaborative linkage grant, 2004-2006
IN CONDITIONS OF HYPO MAGNESESEMIA:

- TUMOR GROWTH (LUNG, COLON, MAMMARY) WAS SIGNIFICANTLY INHIBITED
- TUMORS WERE LESS VASCULARIZED
- TUMOR OXIDATIVE DNA DAMAGE WAS HIGHER
- CLEAR-CUT SIGNS OF IMMUNO-INFLAMMATORY RESPONSE

Maier JAM et al. *Nutr & Cancer*, 2007
Wolf FI et al., *Nutr & Cancer*, 2008
Inflammation and cancer

- **EXTRINSIC**
  - INFECTIOUS, INFAMMATORY CONDITIONS

- **INTRINSIC**
  - ONCOGENETIC EVENTS

- Transcription factors (*NF-kB; STAT3; HIFs*)
- Inflammatory cells (*PMN, Eo, Macro*)
- CHEMOKINES, CYTOKINES PROSTAGLANDINS:
  - IL-1, TNF, VEGF, CXCL8, CCL2, COX2

CANCER-RELATED INFLAMMATION
CANCER-RELATED INFLAMMATION

Tumour microenvironment

Proliferation, survival, EMT;
angiogenesis and lymphangiogenesis;
migration, invasion, metastasis;
inhibition of adaptive immunity;
Response to hormones and chemotherapeutic agents
Mg deficiency enhanced **metastatic potential** of LLC cells in C57Bl/6 mice

Lung metastatic focus in Mg-deficient mice

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**Magnesium Deficiency Inhibits Primary Tumor Growth But Favors Metastasis in mice.**

*Nasulewicz A.,et al., BBA, 2004*
Magnesium and Tumour treatment

Cisplatin

EGFR mAb: cetuximab

Hypomagnesemia

......does it affect treatment outcome ??

- Normomagnesaemia: 1.9-2.5 mg/dL (0.78-1.03 mmol/L);
- Hypomagnesaemia : < 1.8 mg/dL (0.74 mmol/L);
- NCI-CTCAE grading of symptomatic hypoMg:
  - grade 1 [1.2 mg/dL (0.5 mmol/L)],
  - grade 2 [1.2-0.9 mg/dL (0.5-0.4 mmol/L)],
  - grade 3 [0.9-0.7 mg/dL (0.37-0.29 mmol/L)],
  - grade 4 [<0.7 mg/dL (< 0.29 mmol/L)];

2FI Wolf, European Mg Workshop, May 014
Magnesium and Tumour treatment:

Clinical Cancer Research

Early Magnesium Reduction in Advanced Colorectal Cancer Patients Treated with Cetuximab Plus Irinotecan as Predictive Factor of Efficacy and Outcome
Bruno Vincenzi, Daniele Santini, Sara Galluzzo, et al.

Overall Survival (months)

Time to Progression (months)

B

P<0.0001

Magnesium reduction > 20% (# patients: 25)

Magnesium reduction ≤ 20% (# patients: 43)

P=0.021

Magnesium reduction > 20% (# patients: 25)

Magnesium reduction ≤ 20% (# patients: 43)
Magnesium and Tumour treatment:

**Incidence and risk of hypomagnesemia in advanced cancer patients treated with cetuximab: A meta-analysis.**


*The study concluded that cetuximab is associated with a significant risk of hypomagnesemia in patients with advanced cancer receiving concurrent chemotherapy.*

**Clinical relevance and utility of cetuximab-related changes in magnesium and calcium serum levels.**


“As hypomagnesemia was more prominent in patients receiving platinum agents, magnesium measurements may be advised in these patients. In mCRC patients treated with cetuximab, day-14 magnesium serum levels correlated with treatment efficacy.”
Magnesium and Tumour treatment:

Association of hypomagnesemia with inferior survival in a phase III, randomized study of cetuximab plus best supportive care versus best supportive care alone: NCIC CTG/AGITG CO.17


Conclusions: In contrast to prior reports, cetuximab-induced hypomagnesemia was associated with poor OS, even after adjustment for grade of rash.
Conclusions

- Magnesium affects all steps of carcinogenesis and tumour growth
  - Low magnesium enhances neoplastic transformation
  - Low magnesium inhibits tumour growth but favors metastasis
  - Hypomagnesemia is a consequence of nephrotoxic therapeutic drugs
  - Its role as predictor factor of therapeutic efficacy or as chemotherapy enhancer is debated
Magnesium and Tumour:

From bedside back to Bench

ION CHANNELS AND MAGNESIUM HOMEOSTASIS
Ion channels in tumours

Regulate:
- Proliferation
- Invasion
- Chemoresistance

Hence......

*Inhibition of Ion channels can be exploited as a New Therapeutic strategy*
TRPM7 in cancer

Functions:

Regulates cell proliferation

Metabolic reprogramming

Migration
TRPM7 and metabolic reprogramming

Cancer cells: Warburg Effect (Aerobic glycolysis)

Proliferating cells: Metabolic reprogramming

High glucose consumption, Macromolecules biosynthesis, DNA synthesis, Cytoskeletal remodelling

FI Wolf, European Mg Workshop, May 2014
Kv11.1 inhibition causes:

- Retarded Cardiac repolarization
- Increase QT interval
- Leading to "torsade de points"
- Ventricular fibrillation
- Hepatotoxicity

F. Wolf, XIII IMS Merida, 2012

TRPM7 metabolism and proliferation

Evidence that TRPM7 is required for breast cancer cell proliferation

Arnaud Guilbert, 1* Mathieu Gautier, 1* Isabelle Dhennin-Duthille, 1 Nathalie Haren, 1 Henri Sevestre, 1,2 and Halima Ouadid-Ahidouch 1
1 Laboratoire de Physiologie Cellulaire et Moléculaire, JE 2530: Canaux Ioniaques dans le Cancer du Sein, Faculté des Sciences, and 2 Service d’Anatomie Pathologique, Centre Hospitalier Universitaire Nord, Amiens, France

Table 1. Correlation between TRPM7 expression and tumor grade

<table>
<thead>
<tr>
<th>Tumor Grade</th>
<th>TRPM7 Overexpression</th>
<th>n</th>
<th>χ²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade I</td>
<td>45.4%</td>
<td>11</td>
<td>0.5051</td>
</tr>
<tr>
<td>Grade III</td>
<td>60%</td>
<td>10</td>
<td></td>
</tr>
</tbody>
</table>

Correlation between melastatin transient receptor potential (TRPM7) expression and tumor grade in 21 patients by χ² analysis is shown. A significant statistical correlation is identified when the returned P was < 0.05.

Transient receptor potential ion channel Trpm7 regulates exocrine pancreatic epithelial proliferation by Mg²⁺-sensitive Socs3a signaling in development and cancer

Nelson S. Yee 1,2,4, Weiqiang Zhou 1,2,4,5 and I-Chau Liang 1,2,4,5
High Expression of Transient Receptor Potential Channels in Human Breast Cancer Epithelial Cells and Tissues: Correlation with Pathological Parameters

Isabelle Dhennin-Duthille1, Mathieu Gautier1, Malika Faouzi1, Arnaud Guilbert1, Marie Brevet1,2, David Vaudry2, Ahmed Ahidouch1,4, Henri Sevestre1,2 and Halima Ouadid-Ahidouch1
TRPM and migration

EGF enhances the migration of cancer cells by up-regulation of TRPM7
Haixia Gao\textsuperscript{a,b}, Xingjuan Chen\textsuperscript{a,b}, Xiaona Du\textsuperscript{a,b}, Bingcai Guan\textsuperscript{a,b}, Yani Liu\textsuperscript{a,b}, Hailin Zhang\textsuperscript{a,b,*}
\textsuperscript{a} The Key Laboratory of Neural and Vascular Biology, Ministry of Education, Hebei Medical University, Shijiazhuang, PR China
\textsuperscript{b} The Key Laboratory of Pharmacology and Toxicology for New Drugs, Department of Pharmacology, Hebei Medical University, Shijiazhuang, PR China

TRPM7 regulates polarized cell movements
Li-Ting SU\textsuperscript{*1,2}, Wei LIU\textsuperscript{1}, Hsiang-Chin CHEN\textsuperscript{*}, Omaya GONZÁLEZ-PAGÁN\textsuperscript{*}, Raymond HABAS\textsuperscript{1} and Loren W. RUNNELS\textsuperscript{1,3}
\textsuperscript{1} Department of Pharmacology, Robert Wood Johnson Medical School, 675 Hoes Lane, Piscataway, NJ 08854, U.S.A., and \textsuperscript{3} Department of Biology, College of Science and Technology, Temple University, 1900 North 12th Street, Philadelphia, PA 19122, U.S.A.
TRPM7 expression in breast cancer

TRPM7 Is Required for Breast Tumor Cell Metastasis


*Published OnlineFirst August 7, 2012; DOI:10.1158/0008-5472.CAN-11-3863*

Cancer Research

Tumor and Stem Cell Biology

F. Wolf, European Mg Workshop, May 2014
TRPM7 expression in cancer

TRPM7 Is Required for Breast Tumor Cell Metastasis


A. Cell proliferation
   - Control
   - TRPM7 shRNA

B. Cell viability
   - Control
   - TRPM7 shRNA

C. Tumor growth in Rag2−/−IL2rg−/− mice
   - Control
   - TRPM7 shRNA

D. Tumor growth in Rag2−/−IL2rg−/− mice

G. Histological section

Published OnlineFirst August 7, 2012; DOI: 10.1158/0008-5472.CAN-11-3863
TRPM7 mediates breast cancer cell migration and invasion through the MAPK pathway

Xiaojing Meng a,1, Chunqing Cai a,1, Jiguo Wu a, Shaoxi Cai b, Changsheng Ye c, Haiyang Chen a, Zhengduo Yang d, Hongqiang Zeng a, Qiang Shen d,*, Fei Zou a,*

a Department of Occupational Health and Occupational Medicine, School of Public Health and Tropical Medicine, Southern Medical University, Guangzhou, China
b Department of Respiratory Medicine, Nanfang Hospital, Southern Medical University, Guangzhou, China
c Breast Center, Nanfang Hospital, Southern Medical University, Guangzhou, China
d Department of Clinical Cancer Prevention, The University of Texas MD Anderson Cancer Center, Houston, TX, USA

European Journal of Cancer (2013) 49, 3694–3707

Available at www.sciencedirect.com

SciVerse ScienceDirect

Transient receptor potential melastatin 7 is involved in oestrogen receptor-negative metastatic breast cancer cells migration through its kinase domain

A. Guilbert a,d, M. Gautier a,d, I. Dhennin-Duthille a,d, P. Rybarczyk a, J. Sahni b, H. Sevestre a,c, A.M. Scharenberg b, H. Ouadid-Ahidouch a,*
TRPM7 over-expression in ductal pancreatic carcinoma

Transient receptor potential melastatin-related 7 channel is overexpressed in human pancreatic ductal adenocarcinomas and regulates human pancreatic cancer cell migration

Pierre Rybarczyk¹, Mathieu Gautier¹, Frédéric Hague¹, Isabelle Dhennin-Duthille¹, Denis Chatelain², Julie Kerr-Conte³, François Pattou³, Jean-Marc Regimbeau⁴, Henri Sevestre¹,² and Halima Ouadid-Ahidouch¹

**TRPM7** regulates cell migration by a Mg²⁺-dependent mechanism.

**TRPM7** is a promising biomarker of PDAC progression and prognosis.
**TRPM7 as a therapeutic target**

**SiRNA or KO** inhibits cell proliferation / migration

*KO cells can survive only in 30 mM Mg$^{2+}$*

**Chemical inhibition**

Co-exammine, APB
Imipramine / Quinidine
Waixenicin (*Zierler, JBC, 2011*)
NS8593 -SCCa-Ki (*Chubanov, BJP, 2012*)
The Role of Waixenicin A as Transient Receptor Potential Melastatin 7 Blocker.

Kim BJ, Nam JH, Kwon YK, So I, Kim SJ.

Epidemiologic data: TRPM7 polymorphism and Ca\(^{2+}\)/Mg\(^{2+}\) in colon carcinogenesis; (Dai, 2007)

TRPM7 and **proliferation** in human **head and neck carcinoma** cells (Jiang CR2007)

TRPM7 suppression induced **apoptosis** in **gastric cancer** (Kim, CS2008)

TRPM7 is required for **breast cancer** cell **proliferation**; overexpressed in grade III breast cancer samples (Guilbert, AJPCP 2009)

TRPM7 regulates the **migration** of human **nasopharyngeal carcinoma** cell (Chen Cell Ca 2010)

Up-regulation of TRPM7 by EGF enhances the **migration** of cancer cells (Gao cell Ca 2011)

TRPM7 in human **breast ductal adenocarcinoma**: prognostic factor (Dhennin-Duthille CPB 2011)

TRPM7 has an important role in the **growth and survival** of **gastric cancer** cells (Kim CJPP 2012)

TRPM7 regulates cell **migration** in human **pancreatic ductal adenocarcinoma** (Rybarczyk IJC 2012)

TRPM7 is required for **breast tumor** cell **metastasis**. (Middelbeek CR 2012)

TRPM77 activated by Ca\(^{2+}\)/Mg\(^{2+}\) promotes **proliferation** of **prostate cancer** cells (Sun JBC 2013)

TRPM7 mediates **breast cancer** cell **migration and invasion** (Meng CL 2013)

TRPM7 is involved in **EMT** in **breast cancer** cells (Davies O 2013)

TRPM7 is involved in ER-metastatic **breast cancer** cells **migration** (Guilbert EJC 2013)
Magnesium transporters in cancer: New raising star

• PRL-2 is key contributors to metastasis in several human cancers
• PRL-2 is overexpressed in breast cancer
• PRL-2 regulates intracellular magnesium levels by forming a functional heterodimer with the magnesium transporter CNNM3
• CNNM3 is not a phosphorylated substrate of PRL-2, the interaction occurs through a loop unique to the CBS pair domains
Magnesium transporters in cancer: New rising star

- PRL-2 knockdown results in a substantial decrease of cellular magnesium influx
- CNNM3 association is important for conferring transforming activities
  in human breast
- cancer tissues showing that CNNM3 levels correlate positively with both PRL-2 expression and the tumor proliferative index.
Magnesium in carcinogenesis
Biochemical, in vitro data
Conclusion

✓ Ion channels are able to affect tumour cell behaviours

✓ TRPM7 is essential component of metabolism, proliferation and invasion

✓ It is overexpressed in some tumours

✓ It can be utilized as a **prognostic factor**

✓ It can be exploited as **therapeutic target**
We propose....

To include the

«ion channel signature»

as a promising strategy for the treatment of cancer

How far, How close........

FI Wolf, European Mg Workshop, May 2014
Other Magnesium transporters or Mg transport-Related protein are emerging

Further research from bench to bedside is required for identifying the most efficient strategy to target magnesium homeostasis as novel therapeutic strategies.
Istituto di Patologia generale
Chairman Prof. Achille Cittadini

Valentina Trapani
Alessandro Sgambato
Alma Boninsegna
Daniela Arduini

INRA Clermont-Ferrand, France
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Jeanette Maier