From magnesium to magnesium transporters in cancer: TRPM7, a novel signature in tumour development

Valentina Trapani, Daniela Arduini, Achille Cittadini, Federica I. Wolf
Istituto di Patologia Generale, Facoltà di Medicina e Chirurgia “A. Gemelli”, Università Cattolica del Sacro Cuore, Largo F. Vito 1, 00168 Rome, Italy
Correspondence: Federica I. Wolf. Istituto di Patologia Generale, Facoltà di Medicina e Chirurgia “A. Gemelli”, Università Cattolica del Sacro Cuore, Largo F. Vito 1, 00168 Rome, Italy
<federica.wolf@rm.unicatt.it>

Abstract. Magnesium availability affects many cellular functions that are critical for tumour growth and spreading, such as proliferation, metabolism and angiogenesis. In vivo, magnesium deficiency, and the resulting inflammation, can trigger both anti- and pro-tumour effects. Recent experimental evidence indicates that altered expression of the transient receptor potential melastatin, type 7 (TRPM7) epithelial magnesium channel is a frequent finding in cancer cells and human tumour tissues, and correlates with cell proliferation and/or migration. We review the role of TRPM7 in tumour development, with particular regard to its channelling function mediating both Ca2+ and Mg2+ influx, as well as its kinase activity, likely regulating actomyosin contractility. The potential diagnostic and therapeutic applications based on TRPM7 detection and inhibition, are also discussed.

Key words: proliferation, angiogenesis, invasion, metastasis, metabolism, TRPM6, TRPM7

Magnesium biology and tumour growth

Many of the hallmarks of cancer are affected by magnesium availability [1-3]. The most relevant ones to our discussion are proliferation, metabolic reprogramming, neo-angiogenesis and invasion.

Cell proliferation

The relationship between magnesium and cell proliferation has been well established since the late ’70s, although the molecular determinants were not identified until the ’90s [4]. Low magnesium availability inhibits cell cycle progression leading to a G0/G1 arrest through the up-regulation of p27, p21 and p16 [5]. In non-transformed cells, this activation is p53-dependent. Magnesium-dependent growth arrest is reversible: upon reintroducing magnesium, the proliferation rate increases to control levels [5]. Proliferating cells contain more magnesium than resting ones, which implies that growth factors are able to activate magnesium uptake from the extracellular compartment. Recently, the identification of specific magnesium transporters has added new molecular details to support such observations. The transient receptor potential melastatin, type 7 (TRPM7) channel is the gatekeeper of magnesium homeostasis in normal and tumour cells, and is essential for cell viability and growth [6]. In particular, TRPM7 is important for cell cycle progression during G1 phase [7]. TRPM7-mediated Mg2+ influx seems to be associated to receptor-mediated mitogenic signalling along the PI3K/Akt/mTOR protein-translation cascade.
As we discuss in the next paragraph, this pathway orchestrates cell proliferation and metabolic reprogramming among other features of tumour cells.

**Cancer metabolic reprogramming**

Aerobic glycolysis was first described by Otto Warburg in the '20s. Since then, it has become clear that complex metabolic alterations in tumour cells represent a core hallmark of cancer, which supports the anabolic requirements associated with growth and proliferation [1, 2]. All dividing cells consume far more glucose than resting ones, as glycolysis provides extremely rapid ATP synthesis compared to oxidative phosphorylation, in spite of lower efficiency. Many enzymes involved in glycolysis, the Krebs cycle and the respiratory chain depend on magnesium either as an allosteric modulator or a cofactor [10]. Mg²⁺ is involved in the regulation of glucose transport; indeed, diabetes and metabolic syndrome are often associated with alterations of magnesium homeostasis [11].

In addition to rapid energy generation, tumour cells “re-program” their metabolism to sustain increased biosynthesis of macromolecules and maintain an appropriate cellular redox status [12]. Of note, magnesium is an absolute requirement for protein synthesis [13] and redox balance [14]. The PI3K/Akt/mTOR pathway regulates cellular growth and biosynthetic processes. The recent demonstration that the TRPM7 channel (and the TRPM7-mediated Mg²⁺ influx) are required for sustained activation of this pathway proves the link between magnesium uptake and metabolic transitions in tumour cells [8, 9].

In addition to rapid energy generation, tumour cells “re-program” their metabolism to sustain increased biosynthesis of macromolecules and maintain an appropriate cellular redox status [12]. Of note, magnesium is an absolute requirement for protein synthesis [13] and redox balance [14]. The PI3K/Akt/mTOR pathway regulates cellular growth and biosynthetic processes. The recent demonstration that the TRPM7 channel (and the TRPM7-mediated Mg²⁺ influx) are required for sustained activation of this pathway proves the link between magnesium uptake and metabolic transitions in tumour cells [8, 9].

Furthermore, the tumour microenvironment exerts crucial selective pressure on tumour cells. Hypoxia is one of the best studied factors that can direct metabolic adaptation. The activation of hypoxia-inducible factor HIF-1α affects multiple pathways, including those regulating glycolysis, lactate production and lactate/proton extrusion, and angiogenesis [15]. Interestingly, in hippocampal neurons, anoxia induces an increase in intracellular magnesium concentration via TRPM7 [16]. These observations seem to support the hypothesis that magnesium uptake could be involved in hypoxia-mediated signals, with possible implications for tumour metabolism.

In summary, magnesium might likely be the common denominator of the three basic needs of all cancer cells, *i.e.* energy, protein synthesis and redox balance; experimental evidence points to an important role for TRPM7 and the PI3K/Akt/mTOR pathway, as foreseen by Harry Rubin [12].

**Neo-angiogenesis**

Neo-angiogenesis is essential for tumour growth and spreading. Magnesium can affect many steps of the complex angiogenic process [17]. Magnesium deficiency inhibits both proliferation and migration of endothelial cells; however, the role of TRPM7 is highly dependent on endothelial cell type [18, 19]. In microvascular cells, which are the real protagonists of neo-angiogenesis, TRPM7 downregulation mimics the effects of low magnesium [18].

*In vivo*, mice receiving a magnesium-deficient diet developed tumours that were significantly less vascularised than tumours grown in control mice [20, 21]. Although these studies remain the only ones specifically addressing the issue, they show that, *in vivo*, the anti-angiogenic effects of magnesium deficiency overcome the pro-angiogenic consequences, as low magnesium availability hinders the formation of new vessels.

**Metastasization**

Malignant tumours are characterised by the ability to metastasize. Magnesium availability also affects metastatic spread, as proven by experimental studies on mice. Mice subcutaneously transplanted with Lewis lung cancer cells, and receiving a magnesium-deficient diet, developed smaller primary tumours, but more metastatic foci in the lung, than mice having a normal magnesium diet [20]. In mice, a magnesium-deficient diet induces severe hypomagnesaemia (~40% of control mice), but also a strong immunoinflammatory response, observed as neutrophilia and an increase in inflammatory cytokines [20, 22]. There are indications that hypomagnesaemia might also represent a pro-inflammatory condition in humans; indeed, low magnesium status correlates with some inflammatory markers [23, 24]. In conclusion, hypomagnesaemia, by inducing a pro-inflammatory condition, might create a microenvironment that favours tumour metastasization.

Translating these findings to clinical settings is not straightforward. Cancer patients often develop hypomagnesaemia following therapy.
with nephrototoxic agents, that impair electrolyte re-absorption in the nephron (e.g. cisplatin), or with anti-EGFR monoclonal antibodies (i.e. cetuximab), which inhibit the critical renal magnesium re-adsorption mechanism via the TRPM6 (transient receptor potential melastatin, type 6) channel. Whether hypomagnesaemic cancer patients should be supplemented or not with magnesium is not a trivial matter. On one hand, magnesium deficiency inhibits proliferation, neo-angiogenesis and DNA repair, thus hypomagnesaemia could represent a condition which increases tumour response to treatment. On the other hand, there is a risk that hypomagnesaemia, possibly by inducing an immuno-inflammatory response, might favour metastatic spread, which poses an alarming warning.

**Magnesium transporters in tumours: TRMP7 “Master and Commander”**

In the previous paragraphs we have described how magnesium availability can affect, not only cell proliferation, but also other crucial features of cancer cells, which are ultimately responsible for tumour growth and spreading. Extracellular magnesium is made available to the cell by specific molecules that regulate ion transport through the plasma membrane, eventually controlling the intracellular magnesium content. Thus, tumour cells are likely to modulate transport mechanisms in order to ensure an adequate magnesium supply to support cell growth. This idea has been corroborated and expanded by recent findings, which have proven the role of TRPM7 in the regulation of cancer cell proliferation, as well as migration and invasion.

The TRPM7 channel and its close homologue TRPM6 have been identified as the gatekeepers of cellular and systemic magnesium homeostasis, respectively [25]. Knock-out studies in mice and other species have proved that both TRPM7 and TRPM6 are indispensable for magnesium homeostasis [26, 27] and embryonic development [28, 29].

It must be noted that the TRPM7 channel is permeable to both Mg$^{2+}$ and Ca$^{2+}$. Since the latter is an important second messenger mediating innumerable cell activities, in many cases Ca$^{2+}$ signals mediated by TRPM7 have been thoroughly examined, while Mg$^{2+}$ fluxes have often been overlooked. However, that both Ca$^{2+}$ and Mg$^{2+}$ can cooperate to control the cell response is emerging from different studies. In some settings extracellular Ca$^{2+}$/Mg$^{2+}$ ratio seems to be more important than Ca$^{2+}$ and Mg$^{2+}$ concentrations on their own [30]. One of the first papers reporting an association between TRPM7 expression and cancer refers to an epidemiological study, whereby a polymorphism in the TRPM7 gene conferred an increased risk of developing a colorectal neoplasia. In this study, a low Ca$^{2+}$/Mg$^{2+}$ intake proved to be protective against colorectal cancer development [31]. More recently it has been described that an increase in the serum Ca$^{2+}$/Mg$^{2+}$ ratio promotes proliferation of prostate cancer cells by activating TRPM7 channels. It was concluded that TRPM7 channel has an important role in prostate cancer and the Ca$^{2+}$/Mg$^{2+}$ ratio could be essential for the initiation/progression of this tumour [32].

TRPM7 and the mediated Ca$^{2+}$ influx are essential for proliferation of human retinoblastoma cells [33], head and neck squamous carcinoma cells [34], human pancreatic and human gastric adenocarcinoma cells [35-37]. The molecular pathway involved in the TRPM7-mediated proliferative signal has been described in DT40 lymphoma cells; importantly, this paper proves that the magnesium influx driven by TRPM7 is responsible for cell proliferation, as growth arrest induced by TRPM7-knockout could be rescued by Mg$^{2+}$ supplementation [9]. A number of studies in human breast [38, 39] and pancreatic [40, 41] cancer biopsies have major clinical relevance, because they found a positive correlation between TRPM7 expression and pathological parameters, including Ki67 proliferative index, tumour size in stage III, and patient survival. In view of these findings TRPM7 expression was proposed as a potential diagnostic/prognostic factor.

TRPM7 and its closest homologue TRPM6 display a carboxy-terminal atypical alpha-kinase domain. The relationship between kinase activity and channel function is still unclear. It is possible that the local increase in Ca$^{2+}$ and/or Mg$^{2+}$ concentration, driven by opening of the channel, might serve for the recruitment/targeting of TRPM7 kinase substrates, and thus transmit intracellular signals via phosphorylation of downstream targets [42]. In this context, it must be underlined that known substrates of the TRPM7 kinase include annexin and myosins [43, 44], which are involved in membrane reorganisation and cytoskeletal dynamics, key features of
the invasive phenotype. Indeed, a significant portion of the latest experimental evidence indicates that TRPM7 also regulates cell migration in cancer cells. In human nasopharyngeal and human lung carcinoma cells, TRPM7 controls basal and EGF-induced cell migration [45, 46]. In human pancreatic adenocarcinoma cells, magnesium influx via TRPM7 stimulates cell migration [40, 41]. In a mouse xenograft model of human breast cancer, TRPM7-knockdown interfered with the metastatic potential of triple negative cells; mechanistic investigation revealed that TRPM7 regulated myosin II-based cellular tension, thereby modifying the number of focal adhesions, cell-cell adhesion and polarized cell movement [47]. Whether TRPM7 regulates migration by its channel function or by its kinase domain remains matter of debate. In oestrogen receptor-negative, invasive breast cancer cell lines, the overexpression of the truncated kinase domain form of TRPM7 decreased cell migration, which was ascribed to reduced myosin IIA heavy chain phosphorylation [48]. It has also been suggested that TRPM7 regulates migration and invasion of metastatic breast cancer cells via the MAPK pathway [49]. Epithelial mesenchymal transition (EMT) represents a crucial switch towards an invasive phenotype: in human breast cancer cells, TRPM7 has been shown to take part in this process, together with other Ca2+ channels [50]. Finally, in neuroblastoma cells TRPM7 seems to trigger invadosome formation, i.e. the assembly of specialised structures able to invade and degrade the extracellular matrix [51]. It is intriguing that the latest findings [48, 50, 51] dissociate the role of the cation influx mediated by TRPM7 from that of the alpha-kinase activity: the latter appears to be more relevant to the regulation of cellular tension and adhesion dynamics to allow migration and metastasis (Table 1).

In conclusion, TRPM7 channels are extremely versatile molecules that could influence tumour cell behaviour by modulating both proliferation and plasticity/motility. These functions might be mediated by Mg2+ and/or Ca2+ influx as well as by the alpha-kinase activity and downstream signalling. It remains to be established whether the two faces (channel and enzyme) of these Janus-like proteins are interdependent or rather, perform separate functions. As a second messenger, Ca2+ is involved in the activation of countless cellular functions; Mg2+, in turn, is essential for sustaining metabolic processes and proliferation, but also for transphosphorylations which are integral part of signal transduction. Since several studies have underlined the role of the Ca2+/Mg2+ ratio, it is conceivable that both cations participate in most processes, probably having different roles, involving rapid on/off signals for Ca2+ and more prolonged availability for Mg2+. In this scenario, one should consider Ca2+ and Mg2+ not as antagonistic, but as collaborating players in regulating cell pathophysiology. The importance of magnesium homeostasis in tumour development has been disregarded for decades, often overwhelmed by the encumbering interest in calcium.

Table 1. TRPM7 in cancer: time chart of epidemiological, in vitro and in vivo observations.

<table>
<thead>
<tr>
<th>Year</th>
<th>Study</th>
<th>Detail</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>2007</td>
<td>Epidemiology</td>
<td>role of TRPM7 polymorphism and Ca2+/Mg2+ in colon carcinogenesis</td>
<td>[31]</td>
</tr>
<tr>
<td></td>
<td>EPIDEMIOLOGY</td>
<td>Sustains proliferation in human head and neck carcinoma cells</td>
<td>[34]</td>
</tr>
<tr>
<td></td>
<td>EPIDEMIOLOGY</td>
<td>Suppression induces apoptosis in gastric cancer</td>
<td>[36]</td>
</tr>
<tr>
<td></td>
<td>EPIDEMIOLOGY</td>
<td>Is required for breast cancer cell proliferation; is overexpressed in grade III breast cancer samples</td>
<td>[38]</td>
</tr>
<tr>
<td>2010</td>
<td>TRPM7 regulates</td>
<td>migration of human nasopharyngeal carcinoma cells</td>
<td>[45]</td>
</tr>
<tr>
<td></td>
<td>Up-regulation of TRPM7 by EGF enhances the migration of cancer cells</td>
<td>[46]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRPM7 is a prognostic factor in human breast ductal adenocarcinoma</td>
<td>[39]</td>
<td></td>
</tr>
<tr>
<td>2012</td>
<td>TRPM7 has an important role in the growth and survival of gastric cancer cells</td>
<td>[37]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRPM7 regulates cell migration in human pancreatic ductal adenocarcinoma</td>
<td>[41]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRPM7 is required for breast tumour cell metastasis</td>
<td>[47]</td>
<td></td>
</tr>
<tr>
<td>2013</td>
<td>TRPM7 activated by Ca2+/Mg2+ promotes proliferation of prostate cancer cells</td>
<td>[32]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRPM7 mediates breast cancer cell migration and invasion</td>
<td>[49]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRPM7 is involved in epithelial-mesenchymal transition in breast cancer cells</td>
<td>[50]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRPM7 is involved in ER+ metastatic breast cancer cell migration</td>
<td>[48]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>TRPM7 triggers invadosome formation in neuroblastoma cells</td>
<td>[51]</td>
<td></td>
</tr>
</tbody>
</table>
Fortunately the latest findings provide the molecular tools and the rationale that should clear the way for a deeper appreciation of magnesium.

**Conclusions and future perspectives**

The molecular identification of magnesium transporters has shed some light on the mechanisms whereby magnesium exerts its pleiotropic effects on cell pathophysiology. In particular, it has made it possible to substantiate the role of magnesium in tumour growth and development. The TRPM7 channel appears to act as “master and commander” of the process by affecting proliferation, migration and metastasization. Interestingly, TRPM7 can mediate both Mg$^{2+}$ and Ca$^{2+}$ influx, which raises the possibility that the two cations may concur in the control of essential cellular functions. Indeed, the serum Ca$^{2+}$/Mg$^{2+}$ ratio, rather than the absolute Ca$^{2+}$ or Mg$^{2+}$ concentration, has been identified as a risk factor for developing colon cancer. Data associating higher levels of TRPM7 expression with growth and progression in certain human cancer types seem to validate the use of TRPM7 expression as a novel biomarker for diagnosis and prognosis. Furthermore, recent evidence indicates that pharmacological inhibition of TRPM7 channel activity impairs the growth and motility of some cancer cell types [52, 53]. These findings have significant therapeutic implications, as they point to TRPM7 being a promising target for the development of specific anti-tumour drugs.

**Disclosure**

Financial support: Work supported by the Italian Ministry of Education, University and Research (D.3.2.2013), and PRIN grant number 2007ZT39FN. The contribution of Tavola Valdese OPM-2013 is greatly acknowledged. Conflict of interest: none.

**References**


8. Sahni J, Scharenberg AM. TRPM7 ion channels are required for sustained phosphoinositide 3-kinase signaling in lymphocytes. Cell Metab 2008; 8: 84-93.


40. Yee NS, Zhou W, Liang IC. Transient receptor potential ion channel Trpm7 regulates exocrine pancreatic epithelial proliferation by Mg^{2+}-sensitive Socs3a signaling in development and cancer. *Dis Model Mech* 2011; 4: 240-61.


45. Chen JP, Luan Y, You CX, Chen XH, Luo RC, Li R. TRPM7 regulates the migration of human nasopharyngeal carcinoma cell by mediating Ca^{2+} influx. *Cell Calcium* 2010; 47: 425-32.


