Magnesium and its transporters in cancer: a novel paradigm in tumour development

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ABSTRACT
The relationship between magnesium and cancer is not as simple as could be assumed from the well-established requirement of magnesium for cell proliferation. Basic and pre-clinical studies indicate that magnesium deficiency can have both anti- and pro-tumour effects. In the present review, we briefly outline the new findings on the role of magnesium in angiogenesis and metastatization, and focus on the relationship between tumour cell proliferation and metabolic reprogramming, discussing how magnesium and its transporters are involved in these processes. The role of magnesium in cancer is also critically examined with regard to mitochondrial function, apoptosis and resistance to treatment. Finally, we bring together the latest experimental evidence indicating that alteration in the expression and/or activity of magnesium channels is a frequent finding in cancer cells and human tumour tissues examined to date, and we discuss the potential implications for developing novel diagnostic and therapeutic strategies.

HYPOMAGNESAEMIA IN CANCER PATIENTS: ALLY OR FOE?
Magnesium is involved in a wide variety of biochemical reactions that modulate key cell functions, such as proliferation, differentiation, migration and apoptosis [1]. As a consequence, a possible role for magnesium among the many diverse players orchestrating neoplastic growth has long been postulated [2], but only recently has clinical and experimental evidence been gathered to substantiate this view.

In the attempt to review the multifaceted role of magnesium in tumour development, we start from clinical evidence, as recent data have added molecular details to the well-known occurrence of hypomagnesaemia in cancer patients, and have raised urgent questions for translating these findings into clinical practice. Hypomagnesaemia in cancer patients derives primarily from therapeutic treatments. Antitumour drugs include several nephrotoxic agents that impair electrolyte re-absorption in the nephron, cause increased magnesium excretion and ultimately lead to hypomagnesaemia [3]. In particular, high-dose or prolonged administration of cisplatin causes tubular damage or dysfunction that surfaces in the form of generalized electrolyte wasting, including that of magnesium [4].

More recently, novel targeted anticancer therapies, such the monoclonal anti-EGFR [EGF (epidermal growth factor) receptor] antibodies cetuximab or panitumumab, have also been found to induce hypomagnesaemia [5]. In this case, however, the molecular mechanism was identified as the specific impairment of the transcellular uptake of magnesium through the TRPM6 [TRP (transient receptor potential) melastatin type 6] channel, the critical magnesium re-adsorption mechanism acting at the level of the distal convoluted

Key words: aerobic glycolysis, apoptosis, metabolism, mitochondrial RNA splicing 2 (mrs2), transient receptor potential melastatin 6 (TRPM6), transient receptor potential melastatin 7 (TRPM7).
Abbreviations: EGF, epidermal growth factor; ERK, extracellular-signal-regulated kinase; HIF, hypoxia-inducible factor; HMEC, human microvascular endothelial cell; IL, interleukin; MDR, multidrug resistant; mrsQ, mitochondrial RNA splicing 2; mTOR, mammalian target of rapamycin; PI3K, phosphoinositide 3-kinase; PTEN, phosphatase and tensin homologue deleted on chromosome 10; TRP, transient receptor potential; TRPM6 etc., TRP melastatin type 6 etc.
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tubule [6]. Hypomagnesaemia, which appears in the first days of therapy and returns to normal levels at the end of the treatment, can cause patient discomfort with symptoms ranging from weakness and behavioural disorders to cardiovascular disorders and seizures. When severe, hypomagnesaemia needs to be corrected by adequate oral or intravenous supplementation [7]. On the other hand, it has been observed that cetuximab-associated hypomagnesaemia occurring in metastatic colorectal cancer patients correlates with a significantly better treatment response in terms of increased time to progression and overall survival [8]. Hypomagnesaemia therefore has actually been proposed as an early, simple and inexpensive biomarker of treatment efficacy and outcome in advanced colorectal cancer patients [9,10].

In general, the recognized role of magnesium in sustaining cell proliferation and the possible inhibitory effect of hypomagnesaemia on tumour growth and neo-angiogenesis [11] seem to corroborate the idea that reduced serum magnesium might improve the response to cancer treatment. Moreover, given the importance of magnesium in DNA repair processes [12], hypomagnesaemia might also be considered a good candidate as a radiosensitizer. This concept remains rather speculative owing to the lack of convincing epidemiological and clinical evidence [13].

In conclusion, the pressing question of whether magnesium behaves as an ally or foe in tumour development and the consequent decision to supplement hypomagnesaemic patients or not [14] remains under debate and requires more specific studies on the subject. Together with translational and clinical data, however, it is imperative to gain a deeper understanding of the cellular and molecular mechanisms underlying the plethora of functions that magnesium co-ordinates to guarantee proper cell behaviour, which are deranged in cancer.

**Hypomagnesaemia and Tumour Growth: Biological Background**

It is well established that intracellular magnesium concentrations affect functions directly linked to those identified as the hallmarks of cancer [15,16]; such aspects have been extensively reviewed recently [17]. In this section, we will focus on two key points: angiogenesis and metastatization.

**Neo-angiogenesis**

Neo-angiogenesis is one of the main culprits of tumour growth and spreading. The angiogenic process involves a highly complex and co-ordinated series of events, including increased vascular permeability, matrix degradation, endothelial cell proliferation, migration, survival and differentiation. Many of these steps are affected by magnesium in different ways [11,14]. Recently, the molecular details of some of the crucial angiogenic steps were evaluated in cultured endothelial cells of different origins in relation to magnesium availability [18]. HMECs (human microvascular endothelial cells) are present in the microvasculature surrounding the tumour and are the real protagonists of tumour neo-angiogenesis. It was found that magnesium deficiency inhibits HMEC proliferation and migration, without affecting metalloprotease production and tridimensional organization; moreover, silencing the magnesium TRPM7 (TRP melastatin type 7) channel mimics these effects [18]. Since low extracellular magnesium markedly decreases TRPM7 in this cell type, it was suggested that TRPM7 down-regulation mediates low-magnesium-induced inhibition of cell growth and migration. Other types of endothelial cells [macrovascular HUVECs (human umbilical vein endothelial cells) and endothelial colony-forming cells], despite being all growth-inhibited by low magnesium, behave differently upon TRPM7 silencing. Thus the contribution of TRPM7 to the regulation of cell proliferation seems to be highly dependent on the endothelial cell type. Other features differentiate the endothelial cell response to low magnesium: in macrovascular cells, low magnesium induces higher levels of protease activity [MMP (metalloproteinase)-2 and -9] [19].

Translating the cellular and molecular mechanisms to systemic settings is never straightforward, but animal studies seem to confirm that low magnesium availability jeopardizes the angiogenic switch that triggers the formation of new vessels. Mice held on a magnesium-deficient diet developed tumours that were significantly less vascularized than tumours grown in control mice [20]. Unfortunately this remains the only study on the subject, but seems to indicate that, in vivo, the anti-angiogenic effects of low magnesium prevail on the pro-angiogenic outcome.

**Metastatization**

Malignant tumours not only invade neighbouring tissues, but can also metastasize by lymphatic or haematic routes, reaching distant sites where they can implant and grow to form a new tumour mass. The involvement of magnesium availability in metastatic spread stands again from experimental studies on mice. Mice were subcutaneously transplanted with Lewis lung cancer cells and kept under normal or magnesium-deficient diets. Unexpectedly, 2 weeks after transplantation it was observed that, despite the smaller size of primary tumours, mice on a low-magnesium diet developed more metastatic foci in the lung than controls [21]. It must be pointed out that, under a magnesium-deficient diet, not only do mice develop severe hypomagnesaemia (~40% of control mice), but also show an intense immuno-inflammatory response [neutrophilia and a increase in IL (interleukin)-1, IL-6 and TNF (tumour necrosis factor)] [22]. The
resulting vasodilation, permeabilization, softening of the extracellular matrix, and production of cytokines and growth factors might all be responsible for favouring cell extravasation and implantation in the pulmonary tissue.

Interestingly, hypomagnesaemia can also be considered a pro-inflammatory condition in humans, as demonstrated by the association found between low magnesium status and markers of inflammation such as CRP (C-reactive protein) and total homocysteine [23,24]. The relationship between inflammation and cancer has gained much attention lately, and the presence of inflammation has been proposed as the seventh hallmark of cancer [25]. The role of inflammation in promoting invasion and metastasis is also well recognized [26]. We believe that hypomagnesaemia, by inducing a pro-inflammatory condition, can create a positive microenvironment that favours tumour metastatization. This point urges a reconsideration of the opportunity to supplement hypomagnesaemic cancer patients or not. If on one hand the inhibition of proliferation, neo-angiogenesis and DNA repair might suggest magnesium deficiency as an effective measure to sensitize the tumour response to treatment, on the other the possibility to enhance the metastatic potential throws out a dreadful caveat to the postulated positive effects of hypomagnesaemia. The scenario that old and new findings concur to delineate is highly complex, and the delicate balance between the ‘good’ and ‘bad’ effects of magnesium availability needs to be carefully evaluated in dedicated pre-clinical and clinical studies.

**MAGNESIUM, TUMOUR GROWTH AND METABOLIC REPROGRAMMING**

**Magnesium and cell proliferation**

The relationship between magnesium and cell proliferation is one of the best known aspects of magnesium cellular physiology. Low magnesium availability inhibits cell-cycle progression leading to a G_{2}/G_{1} arrest through the up-regulation of p27 [27,28], p21 [29,30], and p16 [30]. In non-transformed cells, this activation is p53-dependent. Magnesium-dependent growth arrest is reversible: upon reintroducing magnesium, the percentage of cells in S-phase increases and the levels of cell-cycle-inhibitory proteins decrease, which leads to an increase in the proliferation rate [31]. Proliferating cells contain more magnesium than resting ones, and the required amount can be retrieved irrespectively of extracellular availability over a wide range of concentrations [32]. In other words, no proliferation can occur without an adequate magnesium supply. This concept was first postulated by Harry Rubin in his theory of the ‘co-ordinate control’ of cell proliferation [33], where magnesium was viewed as the most likely ‘second messenger’ for regulating the wide variety of reactions involved in the response to growth factors ultimately leading to cell division. On the basis of biochemical parameters, the same author suggested that mTOR (mammalian target of rapamycin) could be the magnesium-sensitive key regulator of protein synthesis associated with proliferative signals [34]. Rubin’s hypothesis has recently found support from experimental evidence showing that TRPM7-mediated magnesium influx influences signalling along the entire PI3K (phosphoinositide 3-kinase)/Akt/mTOR protein translation cascade [35,36]. As we discuss below, this pathway is crucial in intertwining cell proliferation with metabolic reprogramming and other features of tumour cells; thus PI3K/Akt/mTOR signalling might actually be the fundamental pathway that transduces magnesium availability into cell proliferative behaviour.

Magnesium influx through the homologue TRPM6 channel has been associated with cell proliferation induced by EGF via the MEK [MAPK (mitogen-activated protein kinase)/ERK (extracellular-signal-regulated kinase)/ERK] pathway in renal epithelial cells [37,38]. Interestingly, the same authors recently demonstrated that TRPM6 expression is up-regulated by a PI3K/Akt/mTOR pathway and that rapamycin reduces TRPM6 mRNA stability [39].

Summarizing, not only do the latest findings substantiate the concept that magnesium is a key regulator of cell proliferation, but also highlight a direct link between magnesium homoeostasis and cancer cell metabolism.

**Magnesium and cancer metabolic reprogramming**

Metabolic alterations in proliferating tumour cells represent another crucial hallmark of cancer, whose importance has been reappraised lately. In the 1920s, Otto Warburg [39a] described that tumour cells display a high glucose consumption through the glycolytic pathway, even in the presence of oxygen (aerobic glycolysis), which results in increased lactate production leading to acidification. Indeed, all dividing cells consume far more glucose than resting ones, as glycolysis provides extremely rapid ATP synthesis compared with oxidative phosphorylation, in spite of lower efficiency.

Most of the enzymes involved in glycolysis, the Krebs cycle and the respiratory chain depend on magnesium either as an allosteric modulator or a cofactor [40]. Several old reports suggested an ‘association’ (for lack of a more precise term) between glucose transport and magnesium fluxes in and out of the cell in different cell types [1,41]. Such relationship was indirectly supported by the observation that diabetes and the metabolic syndrome often underlie a dysregulation of magnesium homeostasis [42].
Alterations in tumour metabolism, referred to as ‘metabolic reprogramming’, address not only the need for rapid energy generation, but also two other equally important needs: (i) increased biosynthesis of macromolecules, and (ii) tightened maintenance of appropriate cellular redox status [43]. Indeed, in tumour cells, a large part of glucose metabolites are shifted to catabolic processes, leading to macromolecules and NADPH synthesis. For example, an inactive isoform of pyruvate kinase (PKM2) is expressed by many tumour cells; by slowing glycolysis, this isoenzyme allows carbohydrate metabolites to enter other subsidiary pathways (e.g. pentose phosphate pathway), which generate macromolecule precursors and reducing equivalents such as NADPH [44]. NADPH is a key molecule with a two-fold function: on one hand, it provides reducing power essential for macromolecular biosynthesis and, on the other, by supporting glutathione and thioredoxin reduction, it controls redox status. Since low magnesium induces oxidative stress [45], hypomagnesaemia could enhance the pro-oxidant activities of tumour cells.

It was not until recently that the molecular pathway that links magnesium uptake to cell growth and proliferation, and possibly to biosynthetic metabolism, was described. In tumour B-lymphocytes, the TRPM7 channel (and the mediated magnesium influx) is required for sustained PI3K/Akt/mTOR-dependent growth signalling [35], leading to rapid quiescent/proliferative metabolic transitions [36], as predicted by Rubin [34].

The abnormal tumour microenvironment, for example nutrient and oxygen availability and acidification, exerts crucial selective pressure on tumour cells, also concurring with modifications in their metabolic pathways for survival. Hypoxia is the best documented selective force that directs metabolic adaptation. The activation of HIF (hypoxia-inducible factor)-1α regulates a pleiotropic response that affects the expression of genes regulating glycolysis, lactate production and lactate/proton extrusion, angiogenesis, metastasis and iron metabolism [46].

Novel intriguing findings link magnesium uptake via TRPM7 to hypoxia. In a different context, TRPM7 activation was associated with anoxic neuronal death, which, however, was ascribed to the evoked calcium currents through the channel [47]. Recently, TRPM7-mediated magnesium influx was also studied in an analogous setting. Zhang et al. [48] showed that anoxia induces an increase in the intracellular magnesium concentration in hippocampal neurons. As TRPM7 channels are ubiquitously expressed, it would be very interesting to generalize these findings to other tissue types and to investigate the relationship between hypoxia-mediated signals and magnesium uptake with particular regard to the discussed implications for tumour metabolism. Of note, HIFs interact with the canonical metabolic PI3K/Akt/mTOR signalling pathway [49], which is magnesium-sensitive, as discussed above.

In summary, both genetic alterations and the unique biochemical microenvironment participate in the metabolic reprogramming of tumour cells: classical oncogenic (e.g. c-myc) or tumour-suppressing [e.g. p53 or PTEN (phosphatase and tensin homologue deleted on chromosome 10)] signalling exhibit cross-talk with the best-characterized pathways governing the metabolic phenotype, i.e. HIF-1α and PI3K/Akt/mTOR [43]. Figure 1 schematically summarizes the major mechanisms that converge to alter core cellular metabolism in proliferating tumour cells and highlights cross-talk between multiple pathways and the possible regulatory role of magnesium.

**MAGNESIUM AND MITOCHONDRIA: THE ROLE IN APOPTOSIS**

The Warburg effect was originally proposed to be a result of a permanent impairment of oxidative metabolism [39a]. However, the role of mitochondria in tumour cells has been controversial, since many tumour cell lines with high proliferative rates do not have defects in mitochondrial respiration [50]. Moreover, an association of the lactic acidosis response with good survival outcomes has been found, which may relate to the role of lactic acidosis in directing energy generation toward aerobic respiration and utilization of other energy sources via inhibition of glycolysis [51]. This proves further that tumour cells need to maintain functional mitochondria to reprogramme their metabolism in the ever-changing microenvironment.

Much more than the powerhouse of the cell, mitochondria lie at the centre of essential cellular processes. Beside ATP production and glucose metabolism, they also participate in ROS (reactive oxygen species) generation, the synthesis of metabolites that serve as building blocks for biomolecules and, not least, apoptosis. Above we discussed how most of these processes depend on magnesium and contribute to cancer initiation and progression. In this section, we focus on the role of mitochondria in the apoptotic cascade with particular regard to the contribution of magnesium in this process.

Several *in vitro* studies have reported an early increase in intracellular cytosolic magnesium following both the extrinsic and intrinsic induction of apoptosis [52–54]. Interestingly, the source of intracellular magnesium was hypothesized to be in the mitochondria [53]. Unfortunately, the lack of sensitive, specific and reliable techniques to measure magnesium in the cellular environment has always hindered a detailed study of the subcellular distribution of magnesium and has generated a plethora of often conflicting findings on magnesium content and localization. However, the concept of mitochondria as intracellular magnesium stores has gained much appeal thanks to experimental evidence showing
that, upon mitochondrial depolarization, mitochondrial magnesium release can occur following calcium release and before ATP hydrolysis [55]. It is noteworthy that the same authors have recently demonstrated that glutamate administration to rat hippocampal neurons triggers an excitotoxic pathway whereby calcium accumulation in the mitochondria is required for magnesium release from the organelles [56].

The existence of a mitochondrial magnesium-specific channel further corroborates these findings. The mrs2 (mitochondrial RNA splicing 2) protein is a homologue of the bacterial transporter CorA and is present on yeast and mammalian mitochondrial inner membranes [57]. Mrs2 mediates a high-capacity magnesium influx into mitochondria driven by the inner membrane potential [58] and is essential for the maintenance of respiratory complex I and cell viability [59]. As the activity of mrs2 is dependent on the mitochondrial membrane potential, it might be hypothesized that magnesium stored in the organelles might be released through the channel upon depolarization [55,56].

Intriguingly, mrs2 expression has been associated with resistance to drug-induced apoptosis in cancer cells. Mrs2 transfection was shown to confer an MDR
The molecular nature of the long-postulated mechanisms of magnesium transport did not begin to be unveiled until the late 1990s, when the TRPM6 and TRPM7 channels were identified as the gatekeepers of cellular and systemic magnesium homoeostasis [65]. In particular, TRPM7 was found to be indispensable for cell survival and growth: magnesium supplementation rescued the growth arrest and cell lethality that otherwise resulted from inducible knocking out channel expression [66]. Knockout studies in mice and other species proved that both TRPM7 and TRPM6 are indispensable for magnesium homoeostasis [67,68] and embryonic development [69–72].

In the course of the last decade, several other magnesium transporters have been identified [73] and studies to understand their specific biological role are still in progress. The emerging picture of magnesium homoeostasis is correspondingly becoming more and more complex, which strongly implies the importance of this cation in critical cellular processes.

In comparison with their normal counterparts, tumour cells are notoriously less dependent on the extracellular environment in terms of their requirement for growth factors, adhesion to substrate etc. They also seem to be more self-sufficient with regard to their need of bivalent cations, in particular magnesium. Early findings have demonstrated that several transformed cells become able to proliferate at an extracellular magnesium concentration that does not allow the growth of their normal counterparts [74–76]. We have confirmed that, although the proliferation of normal cells is inhibited in magnesium-depleted medium (from 1.0 down to 0.1 mM), the growth of tumour cells remains unaffected in similar conditions [28]. The absolute requirement of magnesium for cell growth implies that in tumour cells the regulation of magnesium transport must be more efficient to guarantee sufficient magnesium availability and to sustain cell proliferation.

The idea that ion channels may contribute to all of the pathophysiological features that define malignant growth is neither new nor undocumented [77]. Most relevant in this context are the findings that altered expression of one or more TRP channels has been found in several types of cancer and has an involvement in tumour development and progression [78,79]. As for magnesium channels, most of the scrutiny has involved the role of TRPM7 in enhancing cell proliferation and/or migration. One study has also reported an increased risk of developing a colorectal neoplasmia associated with a polymorphism in the TRPM7 gene [80].

TRPM7 seems to be required for proliferation of several types of tumour cells, including leukaemia [36,81], retinoblastoma [82], and carcinoma cells of pancreatic [83,84], breast [85], gastric [86] and head and neck [87] origins. Increased TRPM7 expression was found in human breast [85,88] and pancreatic [83,89] adenocarcinoma tissues, where it correlated with clinicopathological parameters, such as tumour grade, the Ki67 proliferation index and patient survival [88,89]. Table 1 summarizes the results available on TRPM7 expression and activity in tumour cells and tissues.

Growth arrest, rather than apoptosis induction, seems to be responsible for the reduced proliferative rate of cancer cells where TRPM7 activity is disrupted by gene knockout [36], specific channel inhibition [81] or RNA interference [84], although some studies also detected increased apoptosis [87]. This discrepancy might be due to tissue-specific effects. In general, the G2/G1 arrest induced by TRPM7 deficiency is more in line with the well-established magnesium requirement for progression through the cell cycle. At this point, it must be noted that magnesium influx via TRPM7 was only unequivocally shown to be responsible for cancer cell proliferation in some of the studies in which magnesium supplementation rescued the growth arrest induced by TRPM7 disruption [36,83,87]. In the remaining cases, the relevance of
Table 1  TRPM7 expression and its role in cancer development

<table>
<thead>
<tr>
<th>Cancer type</th>
<th>TRPM7 expression in human cancer tissues</th>
<th>Experimental model</th>
<th>Role in tumour development</th>
<th>TRPM7-mediated mechanisms</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colorectal neoplasia</td>
<td>—</td>
<td>Case-control study</td>
<td>Increased risk of adenomatous and hyperplastic polyps</td>
<td>TRPM7 polymorphism; altered Ca(^{2+}/)Mg(^{2+}) ratio</td>
<td>[80]</td>
</tr>
<tr>
<td>Haematopoietic</td>
<td>—</td>
<td>DT40 chicken B-lymphoma cells</td>
<td>Proliferation</td>
<td>PKB/Akt/mTOR pathway; genetic knockout induces quiescence, and Mg(^{2+}) supplementation rescues proliferative arrest</td>
<td>[36]</td>
</tr>
<tr>
<td>Retinoblastoma</td>
<td>—</td>
<td>RBL-1 rat basophilic leukaemia cells</td>
<td>Proliferation</td>
<td>Inhibition by a natural compound induces G(_0/G_1) arrest</td>
<td>[81]</td>
</tr>
<tr>
<td>Pancreatic adenocarcinoma</td>
<td>↑</td>
<td>Human RB cells</td>
<td>Proliferation</td>
<td>Ca(^{2+}) entry</td>
<td>[82]</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>Zebrafish model</td>
<td>Proliferation</td>
<td>Mg(^{2+})-sensitive socs3a signalling</td>
<td>[83]</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>Human pancreatic adenocarcinoma cell lines</td>
<td>Proliferation</td>
<td>Silencing induces replicative senescence</td>
<td>[84]</td>
</tr>
<tr>
<td></td>
<td>↑</td>
<td>Human pancreatic adenocarcinoma cell lines</td>
<td>Migration</td>
<td>Silencing inhibits cell migration without affecting proliferation; Mg(^{2+}) supplementation fully restores migration; expression correlates with patient survival inversely</td>
<td>[89]</td>
</tr>
<tr>
<td>Breast adenocarcinoma</td>
<td>↑</td>
<td>Human breast cancer cell lines, primary cultures of human breast cancerous epithelial cells</td>
<td>Proliferation</td>
<td>Ca(^{2+}) influx; expression correlates with Ki67 or tumour size in stage III</td>
<td>[85,88]</td>
</tr>
<tr>
<td>Gastric adenocarcinoma</td>
<td>—</td>
<td>Human gastric adenocarcinoma cell lines</td>
<td>Silencing induces apoptosis, rescued by high Mg(^{2+})</td>
<td>—</td>
<td>[86]</td>
</tr>
<tr>
<td>Head and neck</td>
<td>—</td>
<td>Human head and neck squamous carcinoma cell lines</td>
<td>Proliferation</td>
<td>Ca(^{2+}) influx, silencing or chemical inhibition arrests growth</td>
<td>[87]</td>
</tr>
<tr>
<td>Lung adenocarcinoma</td>
<td>—</td>
<td>Human nasopharyngeal carcinoma cell lines</td>
<td>Migration</td>
<td>Ca(^{2+}) influx</td>
<td>[90]</td>
</tr>
<tr>
<td></td>
<td>—</td>
<td>Human lung carcinoma cells</td>
<td>Migration</td>
<td>Silencing and/or pharmacological inhibition prevents both basal and EGF-induced migration</td>
<td>[91]</td>
</tr>
</tbody>
</table>

It is also relevant to remember that TRPM7 and its closest homologue TRPM6 have a C-terminal atypical \(\alpha\)-kinase domain. The role of the kinase activity in the regulation of channel function is intriguing. It has been proposed that the opening of TRPM7 channels affects kinase function by causing a local increase in calcium and/or magnesium concentration, which regulates the recruitment/targeting of TRPM7 kinase substrates [97]. In this view, the kinase domain may transmit intracellular signals via phosphorylation of downstream targets, such as annexin I [98], and myosin IIA, IIB and IIC [99], all of which support a role for TRPM7 (and the mediated cation magnesium for TRPM7-dependent proliferation was simply not examined, with the role of calcium influx through the same channel receiving most of the attention.

TRPM7 has also been implicated in the migration of nasopharyngeal [90] and lung [91] cancer cells. In this context, the involvement of calcium has been advocated [92,93], but recently TRPM7 has been shown to regulate migration by a magnesium-dependent mechanism in a pancreatic cancer cell line [89]. Such findings are not surprising, as TRPM7-mediated magnesium influx is known to affect migration in osteoblastic [94,95], endothelial [18] and vascular smooth muscle [96] cells.
TRPM7 and the associated molecular pathways regulating tumour growth and spreading

TRPM7-dependent magnesium entry influences signalling along the entire PI3K/Akt/mTOR protein translation regulatory pathway. TRPM7-dependent magnesium uptake activated by growth factors (GFs), hypoxia and/or other signals can rescue lymphocytes from quiescence to proliferation by increasing aerobic glycolysis. The PI3K signalling cascade appears as the integrating point between magnesium availability and cell proliferative behaviour in terms of metabolism, macromolecular synthesis and, ultimately, cell division. On the other hand, it has been proposed that the opening of TRPM7 channels affects its kinase function by causing a local increase in calcium and/or magnesium concentration, which regulates the recruitment/targeting of TRPM7 kinase substrates. The kinase domain may transmit intracellular signals via the phosphorylation of downstream targets, such as annexin I, and myosin IIA, IIB and IIC, which all support a role for TRPM7 in membrane reorganization and cytoskeletal dynamics necessary for cell migration and invasion. Altered TRPM7 expression and/or activity has been associated with increased proliferation or migration in a number of different cancer cell types. GLUT, glucose transporter; MCT, monocarboxylate transporter.

In conclusion, TRPM7 channels are extremely versatile molecules that could influence tumour cell behaviour by modulating both proliferation and plasticity/motility. Although the former function has been convincingly associated with cation influx, and in particular with magnesium influx, the latter seems to be more dependent on the related ω-kinase activity, but the molecular details linking channel activity to downstream signalling are still missing. Figure 2 provides an integrated picture of the molecular pathways activated by TRPM7 which affect cell features crucial for tumour growth and spreading.

As we briefly mentioned above, the list of magnesium transporters grows longer and longer [73]; unfortunately, their functional characterization lags far behind. Consequently, to the best of our knowledge, no findings exist regarding the altered expression/function of other magnesium transporters in cancer, the only exception being mrs2 [60]. In particular, we believe that the role of TRPM6 in human cancers is an issue worthy of further investigation, given the close functional relationship existing between the two sister channels.

Taken together, following a review of the literature it is apparent that the importance of magnesium homoeostasis in tumour development has been disregarded for decades, often obscured by the encumbering attention for calcium. Fortunately, the latest findings provide the molecular tools and rationale to clear the way for a greater appreciation of magnesium.

CONCLUSIONS AND FUTURE PERSPECTIVES

Over the last few decades, the basis for interest in magnesium as an essential modulator of cell function has moved from biochemical grounds to cellular and molecular evidence that has greatly added not only to our knowledge, but also to our toolbox. In particular, magnesium appears to be crucial for tumour growth and progression. In clinical settings, the assumption that magnesium deficiency may, in principle, potentiate the effects of antitumour radio- and/or chemo-therapy is supported by diverse experimental evidence, but we lack studies that specifically address the issue and correlate the biological effects with clinical implications.

The recently identified molecular determinants of cellular magnesium homoeostasis have brought to light novel pathways whereby magnesium might affect cell behaviour. The relationship between TRPM7 and TRPM6 expression and/or activity and signalling through the PI3K/Akt/mTOR pathway seems to represent the long-sought missing link between magnesium availability and key cell functions deranged in cancer, such as metabolism and proliferation.

In contrast with the classical assumption that magnesium and calcium behave as antagonists, recent findings point to their co-operation in signal transduction. A few relevant studies have been able to dissect such signals and have shown that the two cations act synergistically, as for example in T-cell activation [100] or neuronal glutamate excitotoxicity [56]. It is our hope that upcoming research will clarify the different and not necessarily opposing effects of these two essential divalent cations.

It is being increasingly recognized that the expression and activity of different ion channels might mark and regulate specific stages of cancer progression, as is known for example for K⁺ channels, whose contribution to the neoplastic phenotype ranges from the control of cell proliferation and apoptosis to the regulation of invasiveness and metastatic spread [101]. Findings associating TRPM7 expression to tumour growth and progression seem to include magnesium channels in this picture as well. What is more, recent evidence indicates that pharmacological inhibition of TRPM7 channel...
activity impairs the growth and/or motility of some cell types [81,102,103]. These findings raise the exciting opportunity to exploit this knowledge for therapeutic purposes and to develop novel tools (e.g. blocking antibodies, antisense oligonucleotides, small interfering RNAs, peptide toxins and small organic compounds) to specifically block TRPM7 channel activity in the hope of winning the battle against cancer.

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