

Altered glutamine metabolism in breast cancer; subtype dependencies and alternative adaptations

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Abstract

Cancer cells must alter their metabolism in order to satisfy the demands of necessary energy and cellular building blocks. These metabolic alterations are mediated by many oncogenic changes that affect cellular signalling pathways, which result in sustained cell growth and proliferation. Recently, metabolomics, has received great attention in the field of cancer research and as the essential metabolic pathways that drive tumour growth and progression are determined, the possibilities of new targets for therapeutic intervention are opened. More specifically, as breast cancer is a heterogeneous disease there is growing evidence that differences in metabolic changes exist between molecular subtypes.

In this review, the most recent findings in breast cancer cell metabolism are discussed, with particular emphasis on glutamine and its transporters which is considered one of the key amino acids fuelling cancer growth. Furthermore, the metabolic differences between the molecular subtypes of breast cancer are examined, highlighting the clinical utility for breast cancer diagnosis and treatment.

Key words: breast cancer, metabolic pathways, glutamine, molecular classes

Introduction

One of the hallmarks of cancer ¹, altered metabolic profile, has been demonstrated for many years, and the significance of cancer metabolism and its exploitation for anticancer therapy has recently attracted great attention. Relentless proliferation of cancer cells is metabolically demanding, requiring ATP generation for the necessary energy and production of metabolic intermediates. The latter are the precursors for cellular ‘building blocks’, in the form of nucleotides for DNA/ RNA, amino acids for proteins and fatty acids for lipids. In addition to increasing the amount of metabolites the cells require, cancer cells actively alter the way nutrients are used. This adaptation process has been termed “metabolic transformation” ².

In order to fuel the aberrant proliferation, cancer cells reprogram their glucose metabolism. Glycolysis is normally elevated under anaerobic conditions and is the major source of energy in malignant tumours, even with the presence of oxygen; a phenomenon known as aerobic glycolysis or the Warburg effect ^{3,4}, where glucose is mainly converted to lactate rather than engaging in the mitochondrial oxidative phosphorylation. **Glutamine is the second primary metabolite to nourish cancer cell proliferation.** Although it is a non-essential amino acid in normal cells, glutamine becomes essential to the proliferative neoplastic cells and certain cancer cell types become “addicted” to glutamine as they fail to grow or proliferate in its’ absence ⁵. **Glutamine metabolism not only assists ATP production, it is also essential for biosynthesis of nucleotides, lipid and proteins in addition to its role as a regulator for redox balance ⁶. SLC1A5 (ASCT2) and SLC7A5 (LAT1) are the key cellular glutamine transporters which show higher affinity for their substrates and both are up-regulated in a variety of cancers where their expression pattern is comparable ⁷.**

Changes in glutamine metabolism are controlled by activated oncogenes (e.g. *MYC*) and/or loss of tumour suppressors, e.g. Retinoblastoma (*Rb*), which can enhance the uptake and

utilisation via up-regulation of cellular transporters and enzymes supporting tumour replication and giving rise to aggressive cancer phenotypes^{8 9}.

Breast cancer (BC) is a heterogeneous disease characterised by different morphology, clinical outcome and response to treatment. Based on the gene expression profile, BC can be classified into different molecular subtypes; luminal A (ER and/or PR receptor positive, HER2 negative, low Ki67), Luminal B (ER and/or PR receptor positive, HER2 positive, or HER2 negative with high Ki67), basal-like tumours (mainly triple-negative BC) and HER2 positive (ER/PR negative, HER2 positive). Luminal tumours usually confer good clinical outcome, while the last two subtypes confer shorter relapse free and overall survival^{10, 11}. BC also shows heterogeneity in the metabolic reprogramming including glutamine metabolism. Therefore, understanding the cellular metabolism in different subtypes will help in identifying potential novel therapeutic targets.

This review highlights the metabolic modulations in breast cancer with emphasis on the role of altered glutamine metabolism and glutamine transporters in the different molecular subtypes.

Glutamine metabolism in breast cancer cells

Non-essential amino acids are synthesised endogenously by mammalian cells. However, in tumour cells they become essential in order to meet the needs of rapid cell growth¹². Glutamine is the second most essential nutrient used after glucose for driving tumour cell proliferation and survival¹³ with some cancer cells displaying glutamine addiction, to facilitate cell metabolism and sustain growth. Glutamine can provide the tumour cell with several mechanisms to achieve this (Figure 1). Firstly, glutamine provides the nitrogen required for the biosynthesis of nucleotides and non-essential amino acids¹⁴. In addition, glutamine can replenish the mitochondrial Tricarboxylic acid cycle (TCA) carbon pool via α -ketoglutarate (α -

KG) synthesis and subsequently, α -KG can undergo reductive carboxylation which supports citrate and fatty acid synthesis in stress conditions such as hypoxia^{15,16}.

Alternatively, glutamine can be metabolised by glutaminases to provide glutamate. The latter is a precursor for glutathione (GSH), which is used to reinforce the anti-oxidant response and maintain the redox balance within cancer cells^{17,18}. A further role for glutamine is facilitating the import of essential amino acids including leucine which maintains activation of the mammalian target of rapamycin complex1 (mTORC1), which in turn regulates protein translation and prevents apoptosis in cancer cells¹⁹. Furthermore, in tumours that bear mutations in isocitrate dehydrogenase (IDH1/ IDH2) such as glioma²⁰ and cholangiocarcinoma²¹, mitochondrial glutamine may be a source for 2 hydroxyglutarate (2HG) which competitively inhibits α -KG-dependent dioxygenase. If accumulated, 2HG causes aberrations in DNA and histone methylation that result in increased stem cell marker expression indicating a switch to a stem-like phenotype in cancer cells^{22,23}. It has been found that 2HG can be accumulated in MYC driven BC without IDH mutation²⁴. Both Triple negative (TNBC) and HER2-positive breast cancer have higher levels of glutamine consumption and glutaminolysis compared with luminal subtypes²⁵.

Mutational landscapes control glutamine and glucose metabolism in breast cancer

Glucose and glutamine serves as the primary nutrients to fuel cancer cell proliferation and indeed the rate of their utilisation far exceed the consumption of other nutrients. It is been found that the metabolism of these two nutrients is co-regulated as cancer cells which depend on glycolysis must increase the rate of glutamine consumption to provide α -KG to the TCA cycle for further production of intermediates required for biosynthetic pathways. This reprogramming is controlled by mutations in oncogenes and/or tumour suppressors or more directly by copy number aberrations of metabolic genes themselves²⁶.

The most frequent of these aberrations is TP53 mutations, which occur in approximately 80% of basal-like and HER2-positive tumours²⁷. **Wild type P53 targets cytochrome C oxidase-2 (SCO2), which promotes aerobic respiration, and TP53-induced glycolysis regulatory phosphatase (TIGAR), which inhibits glycolysis. Thus, loss of TP53 function shifts metabolism toward aerobic glycolysis and Warburg metabolism^{28, 29}. In addition, mutations in PI3K pathway, which commonly occur in the luminal subtypes of BC, not only stimulate glycolysis through the growth factor (PI(3)K/Akt/mTOR) signalling pathway³⁰, it also induces glycolysis through the activation and mobilisation of aldolase A from the actin cytoskeleton³¹.**

The oncogene MYC is amplified in approximately 15% of breast cancers particularly in basal-like tumours, with a lower occurrence in HER2-positive and luminal subtypes³². **MYC is known to divert glucose metabolism toward aerobic glycolysis and lactate production. Simultaneously, it has a robust influence in glutamine metabolism as it directly facilitates glutamine uptake by binding to the promoters and inducing the expression of glutamine transporters (e.g. SLC1A5 and SLC38A5). It also indirectly promotes the expression of glutaminase (GLS1) by repressing the expression of miR 23 a/b, miRNAs that repress the expression of this enzyme^{33, 34}. Additionally, MYC- driven ER-negative tumours showed GLS over-expression and 2HG accumulation conferring poor overall survival²⁴. Glutamine uptake can be also regulated by Rb family proteins via controlling the E2F-3 transcription factor, which associates with the SLC1A5 promoter and directly regulates its gene expression where Rb knockout cells exhibit elevated SLC1A5 when compared to Rb wild type cells³⁵.**

Metabolic signature in breast cancer varies among different molecular classes

Recently, metabolic profiling of breast tumours has demonstrated a large difference between malignant and normal breast cells^{36, 37} which has been further established within the different molecular breast cancer subtypes, where ER-negative tumours can be distinguished from those that are ER-positive depending on their metabolic signatures.

Breast cancer cells have been shown to overexpress glucose transporters and glycolytic enzymes involved in the metabolic process. For example, Kanaan et al reported an elevated level of nearly all glycolytic intermediates with strong lactate accumulation in TNBC when compared to ER-positive tumours. However, there was a slight decrease in citrate observed in TNBC, indicating an increase in Warburg metabolism, where glucose-derived pyruvate is shunted to lactate, and citrate is utilised in fatty acid biosynthesis³⁸⁻⁴⁰. Similarly, it has been reported that the highly invasive MDA-MB-231 (TNBC) cell line has much higher glucose consumption rates than MCF7 cells (ER-positive, luminal A)⁴¹.

Glutamine dependent mechanisms also can vary substantially among breast cancer subtypes. TNBCs have increased expression of glutamine metabolic enzymes⁴². However, the highest levels of glutamine metabolism with increased GLS and glutamate dehydrogenase (GLUD) expression is observed in HER2-positive breast cancer, while luminal A tumours have the lowest level of these enzymes²⁵. This is in concordance with higher levels of glutamate and lower levels of glutamine in TNBC and HER2-positive tumours compared with ER-positive tumours suggesting an increase in glutamine consumption and glutaminolysis in these tumours^{38, 39}. Subsequently, glutamine deprivation can reduce the proliferation and progression especially among the most aggressive types of breast cancer.

Glutamine transporters and their relevance to breast cancer

In general, all amino acids, including glutamine, require the aid of selective transporters to be able to cross the plasma membrane. Approximately fourteen amino acid transporters accept glutamine as a substrate. However, none are solely selective for this amino acid as they also transport other neutral, cationic and anionic amino acids. Furthermore, they function either in the influx or efflux of glutamine into/from cells. These transporters belong to four different gene families; Solute carrier family (SLC) SLC1, SLC6, SLC7, and SLC38; namely SLC1A5, SLC6A14, SLC6A19, SLC7A5-9, SLC38A1-3, -5, 7-8 [22].

Table 1 summarises the glutamine transporters along with their functions and relevance to breast cancer.

Reviewing all of the glutamine transporters is beyond the scope of this review, however within the field of breast cancer metabolism, SLC1A5 and SLC7A5 have been attracting particular attention due to their role in supporting tumour metabolism and potential as a therapeutic target within the aggressive sub-classes of BC.

SLC1A5 and SLC7A5 are both expressed in the aggressive subtypes of breast cancer indicating that the two transporters co-operate at the functional level in promoting tumour growth, as SLC1A5 maintains the sodium-coupled influx of glutamine, which preserves the biosynthetic pathway of the rapidly growing cancer cells, whereas SLC7A5 mediates the efflux of this amino acid in exchange with the influx of leucine, an essential amino acid and potent activator of mTORC1 (Figure 1).

It is known that c-MYC enhances the expression of SLC1A5 and SLC7A5 through binding to their promoter regions [39, 40]. Upon glutamine deprivation, activation factor 4 (ATF4), in coordination with MYC, directly activates the expression of both transporters to maintain

intracellular levels of amino acids [41, 42]. High expression of SLC1A5 and SLC7A5 was observed in TN and HER2-positive BC compared with luminal subtypes [20], [43]. Recent work carried out by our group (data unpublished), have shown that SLC1A5 and SLC7A5 proteins are predominantly expressed in the membrane of the more aggressive ER-positive luminal B tumours where it confers a poor patient outcome (Figure 2A and 2B). In addition, cases which exhibited co-expression of both transporters showed a poorer outcome compared to the single expression of each protein. Indeed SLC7A5 is included, along with other immunohistochemical markers, in Mammostrat® test, which predicts recurrence rate of early stage breast cancer patients treated with endocrine therapy ⁴³. In addition, it is shown that this biomarker, along with carcinoembryonic antigen-related adhesion molecules (CEACAM5 and CEACAM6), can act as a predictor of poor response to neoadjuvant chemotherapy in locally advanced breast cancer. Therefore, it will be possible to limit the toxicity of chemotherapy of non-responder patients ⁴⁴.

Other glutamine transporters that have been investigated in breast cancer include SLC6A14 and SLC7A8 which are upregulated in ER-positive but not ER-negative breast tumours ^{45, 46}. In addition, SLC7A7 may be considered a progesterone receptor (PR) target gene, as a study carried out by Leo et al., showed a 6.5 fold expression change within the TNBC cell line MDA-MB-231 after PR-transfection ⁴⁷. Additionally, SLC7A7 along with SLC7A8, SLC7A9 and SLC38A5 are overexpressed in HER2-positive breast cancer cells line ⁴⁸. Moreover, SLC38A1 is upregulated and overexpressed in different breast cancer cell lines, particularly those that are ER-negative, compared with normal breast tissues ⁴⁹. SLC38A2 has not been investigated in specific breast cancer subtypes but it has been found to be upregulated by BRCA1 which is frequently associated with basal-like TNBC ^{50, 51}. Other glutamine transporters have yet to be studied in breast cancer.

Metabolic biomarkers can be exploited to predict breast cancer progression and response to therapy

Various metabolic pathways of cancer cells harbour targets that can be exploited for treatment of malignant diseases, including breast cancer. These agents can deactivate the essential pathways which result in lack of energy and the necessary molecules required for cancer cell growth and survival.

Breast cancer patients with ER-positive and HER2-positive subtypes respond well to endocrine therapies and anti-HER2 agents respectively. However, some cancers exhibit resistance. Moreover, conventional chemotherapy remains the main strategy for treating the aggressive TNBC as there currently lacks targeted therapy for these tumours. Therefore, cellular metabolism in breast cancer may be promising for developing targeted therapies with fewer complications and which may also reverse drug resistance. Metabolic approaches including liquid chromatography-mass spectrometry (LC-MS), gas chromatography-mass spectrometry (GC-MS) and nuclear magnetic resonance (NMR) spectroscopy, can be applied to analyse different metabolites in tumour tissue and plasma in order to identify novel diagnostic and prognostic biomarkers, as well as providing a new anti-cancer agents especially in tumours that lack response to conventional treatment.

Differences in metabolic signatures between ER-negative and ER-positive breast tumours may further guide therapy and predict disease outcome. GLS inhibitor CB-839 for example, is currently being assessed in phase II clinical trials in many solid tumours including in patients with TNBC ⁵². Similarly, targeting glutamine transporters *in vitro* show a considerable inhibitory effect on these highly aggressive breast cancer cells ^{42,53}. Interestingly, TNBC cells are particularly sensitive to the pharmacological inhibition of mTORC1 ⁵⁴. Likewise, a clinical trial showed that trastuzumab-refractory HER2-positive patients can be actively treated with

mTORC1 inhibitors combined with anti-HER2 agents ⁵⁵. Accordingly, targeting SLC1A5 and SLC7A5 may afford a potential role in treating patients with the most aggressive breast cancer subtypes through impeding the import of leucine and the subsequent mTORC1 activation. Thus, designing specific inhibitors, which target this pathway, should be further investigated.

Targeting the transcription factor MYC is another aspect that can be exploited for blocking cancer metabolism which controls metabolic changes in cancer especially for glutamine import and catabolism. It has been challenging to target the pleiotropic MYC therapeutically. However, the small molecule inhibitor, CX-3543, that binds G-quadruplexes which exists in the MYC promoter is currently being tested in phase II clinical trials ^{56,57}.

Targeting metabolic reprogramming can be potentially useful as a strategy to overcome resistance to therapy. It is been found that enhanced Warburg metabolism, via AKT/mTOR/HIF-1 α modulations, is one of the mechanisms for deriving tamoxifen resistance and impairing glycolysis can restore tamoxifen sensitivity in the endocrine-resistant BC cells ⁵⁸. Similarly, Zhao et al found that increased glycolysis in HER2+ cancer cells, through the upregulation of heat shock factor 1 (HSF1) and lactate dehydrogenase A (LDHA) can participate in trastuzumab resistance, as a combination of glycolysis inhibitor and the anti-HER2+ drug inhibited the growth of trastuzumab-resistant breast cancer.

It is important to note that cancer cells exhibit metabolic plasticity which allow them to survive and grow under unfavourable and stressful environments, making this character a major issue facing metabolic targeting of cancer cells. Targeting a particular metabolic pathway may not be sufficient as a viable strategy for cancer treatment as tumour cells could develop resistance through expressing other alternative pathways. For example, metastatic breast cancer shows striking plasticity to adapt their metabolism and growth under different conditions.

To allow exposing to microenvironmental changes which better represent invivo systems, Magnetic Resonance Spectroscopy (MRS) was applied, by Simões et al, in a high-density 3D invitro model. They observed that under glutamine starvation, metastatic breast cancer cells (4T1) showed increased pyruvate carboxylase activity, which catalyses the carboxylation of pyruvate to form oxaloacetate (OAA), while glutamine supply can cause decrease in the activity of this enzyme, as more glutamine is available to replenish the TCA cycle at the level of OAA. This indicates the presence of an alternative pathway into the TCA cycle driven by glutamine and independent of glucose ⁵⁹. Therefore, to obtain a more effective anti-cancer therapy of metastatic breast cancer, both pathways, glycolysis and oxidative phosphorylation, should be targeted. In addition, it can be suggested that the synergistic lethality targeting both glutamine and glucose pathways may prove to be more effective in the treatment of cancer, taking into account the toxic effect of these on normal cells, otherwise this treatment regime may be accompanied with the same types of toxicity caused by conventional chemotherapy.

Conclusion

Breast cancer exhibits disparity among their molecular classes for the relative importance of metabolic pathways. The most prominent is the aggressive metabolic pattern of TNBC and the changes in the glutamine pathway. Studying the metabolic pathways of these breast cancer subtypes can lead to the discovery of candidate biomarkers as well as providing novel agents which can guide to a personalised treatment. Designing metabolic targeting agents with a synergistic lethality may prove to be more effective in cancer treatment. Overall, the integration of metabolomic characteristics of breast cancer with genomic, transcriptomic, and proteomic data is a way forward towards a more comprehensive understanding of the disease progression and response to therapy.

Declaration

The authors declare that they have no competing interests.

Statement of author contributions

RE, writing the review article; AM, EAR, IOE, ARG, MC; contribution to writing and reviewing the article; all authors approved the submitted final manuscript.

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Table1. Glutamine transporters, their functions and relevance to breast cancer

Transporter	Function	Observations in different BC subtypes
SLC1A5	Glutamine influx/efflux	<ul style="list-style-type: none"> Expressed mainly in HER2+ and TNBC Inhibition or knockdown of SLC1A5 decreased tumour cell growth in basal-like cells (HCC1806) <i>in vitro</i> and <i>in vivo</i>, while the growth of MCF7 was unaffected ⁴².
SLC7A5	Glutamine influx/efflux	<ul style="list-style-type: none"> Expressed mainly in HER2+ and TNBC. Applying SLC7A5 inhibitor BCH or knockdown significantly inhibits MDA-MB-231 cell growth ⁵³.
SLC6A14	Glutamine influx	<ul style="list-style-type: none"> Up-regulated in ER+ve BC Treatment with SLC6A14 inhibitor, α-methyl-DL-tryptophan (α-MT), reduced ZR-75-1 cell growth, while it did not affect the growth of MDA-MB-231 ⁴⁵
SLC7A8	Glutamine efflux	<ul style="list-style-type: none"> Up regulated in ER+ve breast cancer and it has the same amino acid substrate like SLC7A5. However, it exhibits relatively lower affinity.
SLC38A1	Glutamine influx	<ul style="list-style-type: none"> SLC38A1 is upregulated and overexpressed in ER-ve cell lines Knockdown of SLC38A1 reduce cell growth of the mouse breast cancer cell line (4T1) ⁴⁹
SLC7A7 SLC7A9 SLC38A5	Glutamine influx Glutamine efflux Glutamine influx/efflux	<ul style="list-style-type: none"> Overexpressed in HER2 positive breast cancer cell lines.
SLC7A6 SLC38A2 SLC38A3 SLC38A7 SLC38A8	Glutamine influx Glutamine influx Glutamine influx/efflux Glutamine influx Glutamine influx	<ul style="list-style-type: none"> Have yet to be studied in breast cancer

Figure legends

Figure1. Role of glutamine in supporting tumour growth

Key: GLUT, Glucose transporter; SLC1A5, Solute carrier family 5 member1; SLC7A5, Solute carrier family 7 member 5; TCA, Tricarboxylic acid cycle; AC-coA, Acetyl coenzyme A; OAA, Oxaloacetate; α -KG, α -Ketoglutarate, LDH, Lactate dehydrogenase; GLS, Glutaminase; GLUD, Glutamate dehydrogenase; mTORC1, mammalian target of rapamycin complex1; 2HG, 2 Hydroxyglutarate.

Glucose and glutamine are the two essential metabolites used to fuel cancer cells. Glutamine plays an essential role in supporting cancer cell growth. Glutamine can act as a nitrogen donor and assist nucleotide biosynthesis and the synthesis of other essential amino acids. Additionally, Glutamine can be metabolised by glutaminases to provide glutamate. The latter, is a precursor for glutathione, which neutralises the reactive oxygen species (ROS). Furthermore, glutamate can be converted to α -KG, which is used to replenish the TCA cycle. α -KG can also be a precursor for the oncometabolite (2HG), which causes methylation specific changes and increases the expression of stem cell markers. Glucose and glutamine are the two essential metabolites used to fuel cancer cells. Glutamine is imported into the cells through glutamine transporters (e.g. SLC1A5) and its efflux is coupled by influx of leucine, through SLC7A5, The amino acid which activates mTORC1 and subsequently supports cancer cell proliferation.

Figure 2. Immunohistochemical expression of SLC1A5 and SLC7A5 in invasive BC

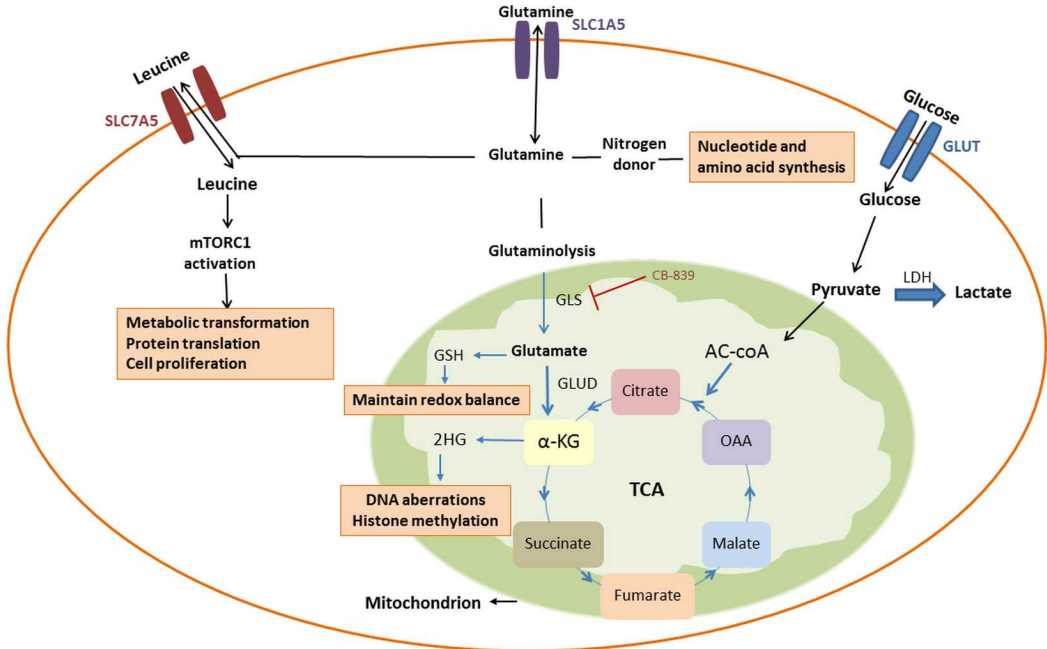
Positive cytomembranous immunoreactivity of glutamine transporters in invasive breast cancer cores. A) SLC7A5, B) SLC1A5.

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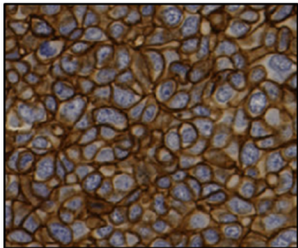
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