LAT1 and ASCT2 Related microRNAs

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The development and progression of colorectal cancer (CRC) have been associated with genetic and epigenetic alterations and more recently with changes in cell metabolism. Amino acid trans-porters are key players in tumor development, and it is described that tumor cells upregulate some AA transporters in order to support the increased amino acid (AA) intake to sustain the tumor additional needs for tumor growth and proliferation through the activation of several signaling pathways. LAT1 and ASCT2 are two AA transporters involved in the regulation of the mTOR pathway that has been reported as upregulated in CRC. Some attempts have been made in order to develop therapeutic approaches to target these AA transporters, however none have reached the clinical setting so far. MiRNA-based therapies have been gaining increasing attention from pharmaceutical companies and now several miRNA-based drugs are currently in clinical trials with promising results.

colorectal cancer amino acid transporters LAT1 ASCT2 miRNAs

1. Introduction

Colorectal cancer (CRC) is one of the most common cancers worldwide, with 1,849,518 new cases in 2018, being the third most common cancer [1]. Currently, CRC accounts for approximately 10% of all diagnosed cancers and it is the world´s second most deadly cancer [2]. CRC is the second most common neoplasia diagnosed in women, and the third in men, being the incidence and mortality approximately 25% lower in woman [2]. CRC development can be modulated by several factors, being the high alcohol consumption, overweigh, physical inactivity, tobacco smoking, diabetes mellitus, age, personal or family history of CRC well established risk factors [3][4]. Although the mortality rates have declined due to the improvement in diagnosis and treatment, CRC still represents one of the most lethal cancer types [3]. Furthermore, metastasis is also found in, approximately, 15–25% of CRC cases at the diagnosis, and increase to 50% during the course of the disease [2][5]. The advances in the pathophysiological and molecular CRC knowledge allowed the increase of the treatment options, but these new therapeutic approaches were proven to be more effective in patients with non-metastatic disease [2]. Thus, it is imperative to clarify the mechanisms involved in disease progression, aggressiveness and metastasis formation in order to improve the patients' follow up and to identify new therapeutic approaches.

2. Amino Acid Transporters Deregulation in CRC: the Impact of LAT1 and ASCT2

It has been nearly a century since the discovery that normal and tumor cells differ in energy metabolism, with tumor cells presenting a higher need of nutrients, being the AA bioavailability crucial to support cell proliferation and

growth [6]. Amino acids can be classified into three groups: (1) essential AA (EAA), if the organism is not able to synthesize them and needs to acquire them from the diet; (2) non-essential AA, if they are synthesized in sufficient quantities by the organism or (3) conditional AA, if are usually nonessential, except in times of illness, trauma or stress were they become conditionally essential [7][8].

In addition to their need in protein synthesis, several amino acids have other roles in supporting cancer development. One example is glutamine, the most abundant AA that participates in energy production, redox homeostasis, macromolecular synthesis and cell signaling [9]. In fact, the commitment of glutamine in the these cell processes makes this AA conditionally essential in conditions characterized by a high proliferation rate, such as cancer, in which endogenous glutamine synthesis is not sufficient to satisfy the cell need [8].

Since AAs are hydrophilic, they need selective transport proteins in order to cross the plasma membrane of the cells. There are approximately two-dozen amino acid transporters in humans, and cancer cells must regulate one or more of these transporters to satisfy their nutrient demand [10]. LAT1 (SLC7A5) is a transmembrane transporter involved in the import of large and neutral AA such as leucine and phenylalanine, in exchange for intracellular AA, such as glutamine [11][12][13]. According to various studies, LAT1 is highly upregulated in multiple human cancers, including gastrointestinal cancers [11][13][14][15]. In fact, Hayase and coworkers found a higher expression of LAT1 in 72.4% of CRC cases when compared to colonic adenoma cases, concluding that LAT1 could be a marker for malignant lesions [11]. Furthermore, Zhang and colleagues also found an association of higher LAT1 expression levels to poorer outcomes and shorter survival in several types of cancer, including CRC [16]. The higher LAT1 expression in cancer cells shows the importance of this AA transporter in the maintenance of AA nutrition in cancer cells [10]. Studies conducted by Elorza and coworkers show that the upregulation of LAT1 is involved in the increase of mTORC1 activity through HIF2α activation, showing a relationship between the hypoxic microenvironment, HIF2α and LAT1 [17]. Furthermore, LAT1 mediates leucine uptake with high affinity, which is a key AA activator of the mTOR signaling pathway [18]. However, for mTOR activation, the functional LAT1 is coupled to ASCT2, another AA transporter involved in glutamine uptake [7].

The ASCT2 (SLC1A5) is expressed in most human tissues including the large intestine and CRC tumor cells, and is essentially responsible for the influx of glutamine inside the cells, inducing asparagine, serine and threonine efflux [19][20][21]. According to Liu and colleagues, ASCT2 expression levels can modulate the migration capacity of CRC cells, being the overexpression of this AA transporters associated with a poorer patients' prognosis [1][22]. In fact, ASCT2 is upregulated in several cancers, including triple-negative breast cancer, CRC, lung cancer, melanoma, neuroblastoma, glioblastoma and prostate cancer [23]. Some studies in glioblastomas and neuroblastoma support the involvement of the activation of c-Myc, n-Myc oncogenes in the inducing of ASCT2 expression [24][25].

Metabolic reprogramming is a well-known hallmark of cancer that has been gaining increasing attention in the last few years due to its importance in cancer cells viability and growth [26]. Cancer associated metabolic reprogramming influences intracellular and extracellular availability of metabolites that will result in alterations in gene expression, cellular differentiation and also in the tumor microenvironment [27]. Glutamine is considered to be

a crucial nutrient for cancer proliferation due to its ability to donate its nitrogen and carbon to several growth-promoting pathways [28]. In 2012, Mootha and colleagues reported that tumor cells have a high necessity of glutamine uptake compared to other AA and, consequently, a glutamine starvation can interfere with tumor metabolism inhibiting tumor proliferation and progression [28]. More recently, Varshavi and colleagues, described a molecular association between CRC that present oncogenic *KRAS* mutation and glutamine metabolism, since these cells exhibit special metabolic phenotypes, including differences in glycolysis, glutamine utilization and AA metabolism [29]. Furthermore, glutamine is described as a signaling factor in the uptake of AA for the activation of mTORC1 [30]. Thus, the upregulation of AA transporters have an important role in the support of the high-level protein synthesis for continuous cancer growth and proliferation [11][31]. The mTOR pathway is well described as deregulated in CRC, and the availability of AA functions as a regulator of this pathway, since a high AA microenvironmental bioavailability induces mTOR activity and consequent biological processes, such as protein translation [32]. Some studies report a relationship between LAT1 and ASCT2, with a two-step mechanism of these AAT being able to regulate mTOR pathway [33][34][35]. Firstly, ASCT2 regulates the intracellular concentration of glutamine, and in turn LAT1 uses this intracellular glutamine as an efflux substrate, in order to regulate the uptake of extracellular leucine, which will lead to an activation of mTOR signaling and consequent induction of cell growth and proliferation [36] (Figure 1). Furthermore, according to Rajasinghe and coworkers, the inhibition of glutamine uptake in proliferating cells, through the inhibition of glutamine transporters LAT1 and ASCT2, results in the inhibition of cell proliferation and induces apoptosis, through the downregulation of the mTOR pathway [34]. Thus, the inhibition of LAT1 and ASCT2 expression levels could represent a promising therapeutic approach for CRC since it would reduce the AA intake, consequently causing mTOR pathway inhibition and compromising cancer cell proliferation.

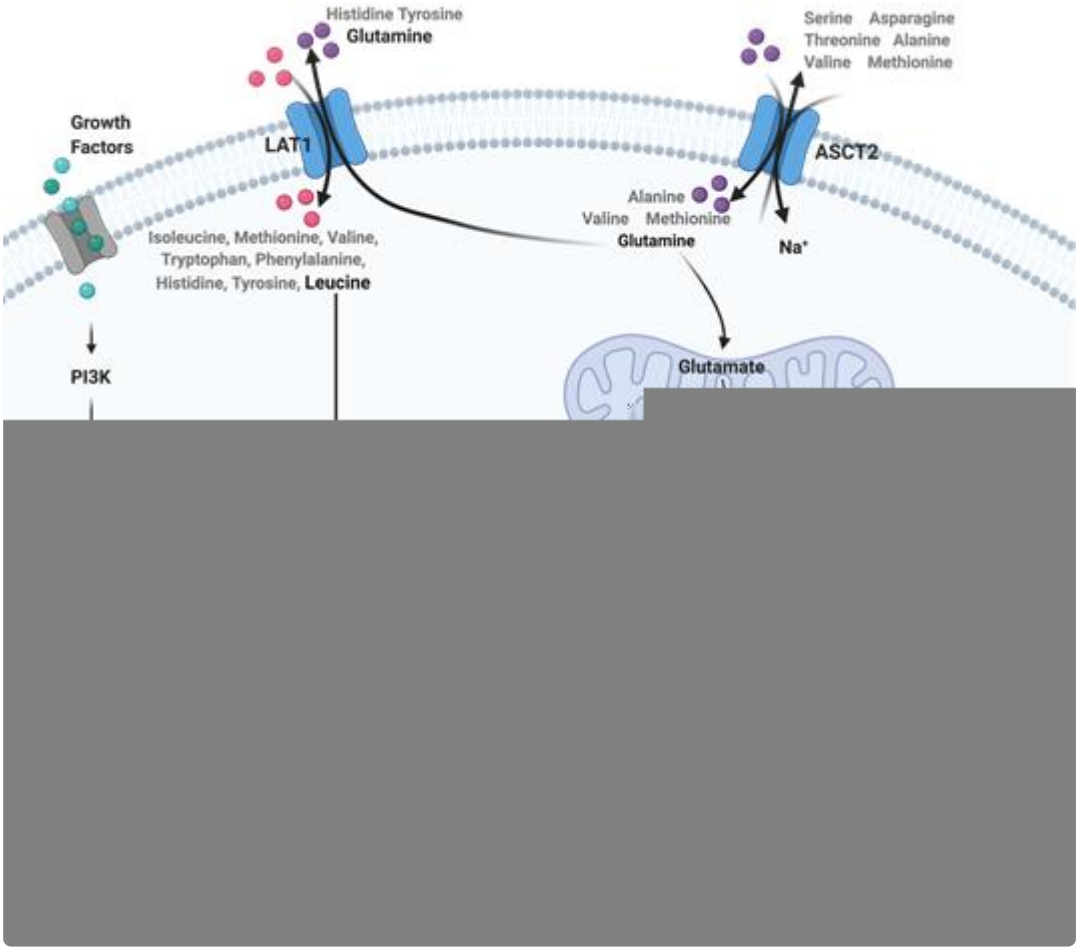

Figure 1. Representation of the interplay between ASCT2, LAT1 and mTOR pathway in colorectal cancer (CRC). This image was created using BioRender.

The use of pharmacologic approaches against LAT1 and ASCT2 in cancers with overexpression of these two AA transporters seems be a promising strategy. In fact, over the last few years there was investment in the development of drugs against LAT1 and ASCT2 [21][34][37][38]. The design of drugs against these two AA transporters usually follows an approach based on substrate analogues, which act as competitive inhibitors [21]. In the case of ASCT2 there are also been developed monoclonal antibodies against its cell surface domains [39]. However, it is imperative to keep in mind that the block of AA transporters could be associated with the upregulation of compensatory and redundant pathways, being crucial an accurate overview of all network involved in the process [40]. In addition to that, there are some limitations in the use of pharmacological inhibitors due to the low affinity for the transporter and low selective capacity observed to cancer cells. Thus, these data highlight the need for a deeper understanding of other therapeutic approaches for the selective inhibition of LAT1 and ASCT2 in CRC.

3. Applicability of microRNAs as Therapeutic Agents

MiRNAs are a family of short non-coding RNAs with a length of approximately 19–25 nucleotides that post-transcriptionally regulate gene expression, with an important role in several biological pathways, including cell proliferation and differentiation [41][42]. MiRNAs can regulate the expression of more than 50% of protein-coding genes by binding to their target mRNA transcript and causing its degradation or translation repression [43]. Regarding their applicability in the clinical setting, a growing number of evidence suggests a significant utility of miRNAs as biomarkers for pathogenic conditions, modulators of drug resistance and as therapeutic agents for medical intervention in almost all human health-related conditions [44][45][46][47]. The pleiotropic nature of miRNAs makes them particularly attractive, both as drugs or drug targets, for diseases with a multifactorial origin and no current effective treatments [48][49]. Overall, the current evidence suggests a viable future for miRNA drugs in diseases with no current effective treatments, such as CRC.

4. miRNAs that target both LAT1 and ASCT2 and their Impact on CRC

From the 33 known miRNA that target both LAT1 and ASCT2, only 16 have already been described in CRC (Table 2). However, in terms of the miRNA:mRNA target interaction with LAT1 and ASCT2, none of the miRNAs have been yet validated for CRC.

Table 2. Selected miRNAs' impact on CRC.

miRNA	Expression	Sample Type	Effect	Reference
Hsa-miR-122-5p	Down	CRC Tissue and cells	Increase in cell proliferation, migration and invasion through the upregulation of CDC25A	Yin 2020 [50]
	Down	CRC Tissues	Upregulation of the PI3K/Akt pathway through upregulation of TRIM29	Asadi 2019 [51]
	Up	CRC liver metastatic tissues	Not described	Liu 2019 [52]
	Up	Serum and HT-29 and SW480	Lymph node metastasis biomarker and cell migration	Qu 2018 [53]

		cell lines	inducer	
	Up	CRC Plasma	Worse prognosis in metastatic patients and shorter RFS and OS in non-metastatic patients	Maiertheler 2017 [54]
Hsa-miR-1224-3p	Up	CRC Tissues	Upregulated in E cadherin positive tissues	Lin 2017 [55]
Hsa-miR-1260a	Down	CRC Serum	Not described	Wang 2017 [56]
Hsa-miR-1260b	Up	HCT116 cells	Chemoresistance to 5-FU through upregulation of PDCD4	Zhao 2018 [57]
	Down	SW480 cells	Downregulated by STAT3-siRNA	Zhang 2014 [58]
	Up	Carcinoma vs adenoma (tissue)	Not described	Slattery 2016 [59]
	Down	CRC Serum	Not described	Zhang 2017 [60]
	Up	DKO-1 cells	Enriched in *KRAS* mutant cells	Cha 2015 [61]
Hsa-miR-1273g-3p	Up	LoVo cells	Proliferation, migration and invasion through activation of ERBB4/PIK3R3/mTOR/S6K2 pathway	Li 2018 [62]

Hsa-miR-1273h-5p	Up	CRC tissues	Not described	Du 2018 [63]
Hsa-miR-149-3p	Down	HCT-8 and HCT-116 cells	Chemoresistance to 5-FU through upregulation of PDK2	Liang 2020 [64]
Hsa-miR-15b-5p	Down	CRC tissues and cell lines	Chemoresistance to 5-FU through upregulation of XIAP	Zhao 2017 [65]
	Up	HT-29 cell line	Cell growth and inhibition of the proapoptotic pathway	Gasparello 2020 [66]
	Down	KRAS mutated CRC tissues vs wild type CRC tissues	Not described	Milanesi 2020 [67]
Hsa-miR-16-5p	Down	CRC tissues and cell lines	Upregulation of VEGFA	Wu 2020 [29]
Hsa-miR-193b-3p	Down	CRC tissues vs adjacent normal tissues	Shorter OS of CRC patients and upregulation of STMN1	Guo 2016 [68]
	Up	CRC tissues	Downregulation of RAD51	Kara 2015 [69]
Hsa-miR-3199	Down	SW620 cell line	Upregulation of SMAD4	Yan 2018 [70]

Hsa-miR-383-3p	Down	CRC tissues and HT-29 and LoVo cell lines	Upregulation of APRIL	Cui 2018 [71]
Hsa-miR-4690-5p	Down	CRC Stool	Not described	Ghanbari 2015 [72]
	Up	CRC tissues	Upregulated in CIMP high/MSI CRC tissues	Mullany 2016 [73]
Hsa-miR-619-5p	Down	CRC tissues vs adjacent normal tissues	Upregulation of MALAT1, lymphovascular invasion perineural invasion, shorter DFS and shorter OS	Qiu 2016 [74]
Hsa-miR-6821-5p	Down	SW480 CSCs vs SW480 wild-type	Not described	Zhou 2019 [75]
	Up	CRC tissues	Not described	Du 2018 [63]
Hsa-miR-6883-5p	Down	TCGA dataset and Cell lines	Upregulation of CDK4 and CDK6 and cell growth stimulus	Lulla 2017 [76]

In table 2 are listed the miRNAs Through the analysis of Table 2 we can observe that some of the miRNAs present opposite results regarding their expression levels, which may be related with the type of biological sample from which their expression levels are analyzed. Regarding their effects on CRC, the deregulation of miR-122-5p, miR-1273g-3p, miR-16-5p, miR-3199, miR-383-3p, miR-619-5p and miR-6883-5p was associated with the upregulation of important players of oncogenic pathways, such as TRIM29, CDC25A, PI3K/Akt, mTOR, VEGFA, MALAT1, SMAD4, STMN1, APRIL and CDK4, with an impact on cell proliferation, invasion and migration. In addition to that, miR-1260b, miR-149-3p and miR-15b-5p were reported as associated with resistance to 50-FU treatment through the upregulation of PDCD4, PDK2 and XIAP, respectively. Moreover, only three miRNAs were associated with clinical endpoints. Higher plasmatic levels of hsa-miR-122-5p were associated with worse prognosis in metastatic

patients and shorter RFS and OS in non-metastatic patients, while lower levels of CRC tissue hsa-miR-193b-3p and hsa-miR-619-5p were associated with shorter OS. Moreover, lower levels of CRC tissue hsa-miR-619-5p were also associated with shorter DFS, lymphovascular invasion and perineural invasion.

Taking this information into consideration, we can conclude that miRNAs that target both LAT1 and ASCT2 play an important role on CRC development and aggressiveness and could be used as potential new therapeutic approaches for this neoplasia, but further studies are needed.

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