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Co-expression of monocarboxylate transporter 1 (MCT1) and its chaperone (CD147) is associated with low survival in patients with gastrointestinal stromal tumors (GISTs)

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Abstract Monocarboxylate transporters (MCTs) have been described to play an important role in cancer, but to date there are no reports on the significance of MCT expression in gastrointestinal stromal tumors (GISTs). The aim of the present work was to assess the value of MCT expression, as well as co-expression with the MCT chaperone CD147 in

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GISTs and evaluate their clinical-pathological significance. We analyzed the immunohistochemical expression of MCT1, MCT2, MCT4 and CD147 in a series of 64 GISTs molecularly characterized for *KIT*, *PDGFRA* and *BRAF* mutations. MCT1, MCT2 and MCT4 were highly expressed in GISTs. CD147 expression was associated with mutated *KIT* (p=0.039), as well as a progressive increase in Fletcher's Risk of Malignancy (p=0.020). Importantly, co-expression of MCT1 with CD147 was associated with low patient's overall survival (p=0.037). These findings suggest that co-expression of MCT1 with its chaperone CD147 is involved in GISTs aggressiveness, pointing to a contribution of cancer cell metabolic adaptations in GIST development and/or progression.

Q3 **Keywords**

Background

The estimated incidence of gastrointestinal stromal tumors (GISTs) ranges from 10 to 20 cases per million annually and the estimated prevalence is around 129 per million (Stamatakos et al. 2009). The majority of GISTs are found in the stomach (50–60%), followed by the small intestine (30–40%), colon and rectum (5–10%) and, rarely, in the esophagus (5%). GISTs are usually solitary tumors that mainly affect individuals over 50 years of age (Chourmouzi et al. 2009) and are generally composed of spindle cells, although sometimes they can display epithelioid features or a mixed pattern (Zhang et al. 2010; Miettinen et al. 2005). These tumors are characterized by a strong, diffuse staining for CD34 (60–70%) and CD117 (>95% of cases) (Blackstein et al. 2006), the latter encoded by the proto-oncogene *KIT* (Mushtaq et al. 2009).

GIST development is associated with activating mutations in the *KIT* gene (85–90%) and, less commonly (around 5%), in *PDGFRA* (Gomes et al. 2008; Martinho et al. 2009), which also encodes a type III tyrosine-kinase receptor. Approximately 10–15% of cases lack mutations in both *KIT* and *PDGFRA* (thus termed wild-type GIST) (Agaimy et al. 2009), and a subset of these wild-type GISTs display activating mutations of the oncogene *BRAF* (Martinho et al. 2009; Agaimy et al. 2009). The mutation profile has an important impact on the response of GIST patients to imatinib, a small-molecule inhibitor of tyrosine kinase. Importantly, patients with *KIT* exon 11 mutations have a better response rate to imatinib and longer overall survival and disease-free interval than patients with exon 9 mutations or wild-type *KIT* (Badalamenti et al. 2007). Nevertheless, it has been reported that patients on imatinib treatment can gain secondary *KIT* mutations, which cause insensitivity to the inhibitor. Resistance to these kinase inhibitors is generally associated with distinctive clinical and molecular features (Gramza et al. 2009).

It was recently proposed that metabolic response measured by fluorodeoxyglucose positron emission tomography (FDG-PET) may be a useful early predictor of GIST response to treatment since there is a lower glucose uptake activity in imatinib-responsive GISTs, while imatinib-resistant GISTs show high glucose uptake capacity (Demetri et al. 2002; Cullinane et al. 2005; Holdsworth et al. 2007). In fact, the high metabolic activity related to intense glycolysis observed in sarcomas decreases and is related to clinical benefit, weeks or months before objective response based on tumor size (Demetri et al. 2002).

It is currently well established that most cancer cells, including GISTs, continually sustain high rates of glycolysis, thus generating high quantities of acids, especially lactic acid. Consequently, the pH of tumor-associated stroma is low, while the intracellular pH is either normal or higher than that of normal tissues. This calls for a transport mechanism capable of transferring acids from cancer cells to the external microenvironment (Chiche et al. 2010). The principal players known to participate in this acid transportation are members of the monocarboxylate transporter (MCT) family, which have a crucial role in conveying lactate across the plasma membrane, thus constituting attractive therapeutic targets (Kennedy and Dewhirst 2010). There is evidence that upregulation of MCTs occurs in many tumors, such as colorectal carcinomas (Pinheiro et al. 2008a), breast cancer (Pinheiro et al. in press) uterine cervix (Pinheiro et al. 2008b) and lung cancer (Koukourakis et al. 2007), among others. There is also an association between lactate derived from tumor cells and cancer progression (Walenta & Mueller-Klieser 2004).

Hitherto, there are no data in the literature regarding the significance of MCT expression in GISTs. Given the aggressiveness of this type of tumor and the potential of MCTs as therapeutic targets, we sought to investigate the expression of MCTs and its chaperone CD147 (EMMPRIN) in a series of molecularly characterized GISTs.

Methods

This study included samples from 51 patients consecutively examined and treated for gastrointestinal stromal tumors (GISTs) at Barretos Cancer Hospital, São Paulo, Brazil, between 2000 and 2008, and 13 patients from Garcia de Orta Hospital, Almada, Portugal, examined and treated between 1994 and 2003. Clinico-pathological data were retrospectively obtained from the files of the hospital medical records. Cases with a history of any previous cancer treatment were excluded. Tumors were classified in accordance with the WHO criteria and the parameters analyzed

included: age, gender, ethnicity, primary tumor site, tumor size, mitotic index, tumor necrosis and risk group as defined by Fletcher and collaborators (Fletcher et al. 2002). Other information included details on ascites metastases, disease recurrence, tumor persistence, distant metastases and cause of death when death occurred. Disease recurrence was defined as tumor detection at a clinical follow-up after 3 months that were free from disease. Tumor persistence was defined as the presence of a palpable tumor seen in clinical follow-ups within the first 3 months. Follow-up data were available for the majority of the patients and were collected through direct interviews with patients or their relatives, and by reviewing the in-hospital patient files. Additionally, information on GISTs molecular status (*KIT*, *PDGFRA* and *BRAF* gene mutations) was also available for patients from Barretos Cancer Hospital (de Oliveira et al. 2011), and performed in the remaining cases (Garcia de Orta Hospital) as previously described by our group (Gomes et al. 2008; Martinho et al. 2009; de Oliveira et al. 2011).

MCT and CD147 immunohistochemistry

MCT detection

Immunohistochemistry was performed in accordance with the avidin-biotin-peroxidase complex principle (R.T.U. VECTASTAIN Elite ABC Kit (Universal), Vector Laboratories, Burlingame, CA, USA), with the primary antibodies for MCT1 (AB3538P, Chemicon International, Temecula, CA, USA), MCT2 (sc-14926, Santa Cruz Biotechnology, Santa Cruz, CA, USA) and MCT4 (AB3316P, Chemicon International, Temecula, CA, USA), all diluted 1:200, as previously described by our group (Pinheiro et al. 2008a).

CD147 detection

Immunohistochemistry was performed using a streptavidin-biotin-peroxidase complex detection system (Ultravision Detection System Anti-polyvalent, HRP, Lab Vision Corporation, Fremont, CA, USA), using a primary antibody raised against CD147 (18-7344, ZYMED Laboratories Inc., South San Francisco, CA, USA), diluted 1:750, as previously described by our group (Pinheiro et al. 2009a).

Immunohistochemical evaluation

MCTs and CD147 immunoreactions were evaluated semiquantitatively using the criteria previously described (Pinheiro et al. 2008a). The immunoreaction extent was scored semiquantitatively as follows: 0: 0% presence of immunoreactive cells; 1: <5% presence of immunoreactive cells; 2: 5–50% presence of immunoreactive cells; and 3: >50% presence of immunoreactive cells. In addition, the intensity of staining was scored semiqualitatively as 0: negative; 1: weak; 2: intermediate; and 3: strong. The final immunoreaction score was defined as the sum of both parameters (extent and intensity), and grouped as negative (score 0 and 1) or positive (3-6). Immunohistochemistry evaluation was performed blindly by two independent observers (AL, CS). Discordant results were discussed under a double-head microscope and a final score was agreed. The presence of plasma membrane staining was not taken into consideration, since the morphology of GIST cells may mislead the interpretation.

Statistical analysis

The available clinical-pathological and immunohistochemical data were analyzed using the SPSS software for Windows, version 18.0 (SPSS Inc., Chicago, IL, USA). All comparisons were examined for statistical significance using Pearson's chi-square (χ^2) test or Fisher's exact test, as appropriate, with threshold for significance p values <0.05. Overall survival curves were plotted using the method of Kaplan-Meier and data were compared by means of the log-rank test. Cases lacking one or more of the clinic-pathological variables were not included in the specific analysis.

Results

A total of 64 samples of GISTs, organized into Tissue Microarrays (TMA) were assessed for MCT1, MCT2, MCT4 and CD147 immunohistochemical expression.

Although MCT and CD147 expression have been described mainly in cell plasma membranes, we found that the expression of these proteins in GISTs is only visualized in the cytoplasm (Fig. 1). Importantly, the majority of positive cases showed both extensive and strong expression of these markers, especially for MCT2 (Fig. 1b). Overall, 89.1% (57/64) of the GISTs were positive for MCT1 (Fig. 1a), 90.3% (56/62) were positive for MCT4 (Fig. 1c), while CD147-positive reactions (Fig. 1d) were observed in 59.4% (38/64) of the cases. All cases were positive for MCT2 (64/64), therefore no additional statistics were performed for MCT2 expression. No associations between the expression of MCT1 and MCT4 and the chaperone CD147 were found (data not shown).

Regarding the molecular profile of the cases (Table 1), we found that CD147 was significantly more frequently expressed in *KIT*-mutated cases, as compared to *PDGFRA*-mutated or wild type cases (p=0.039). However, no difference in mutation location was detected. No differences were

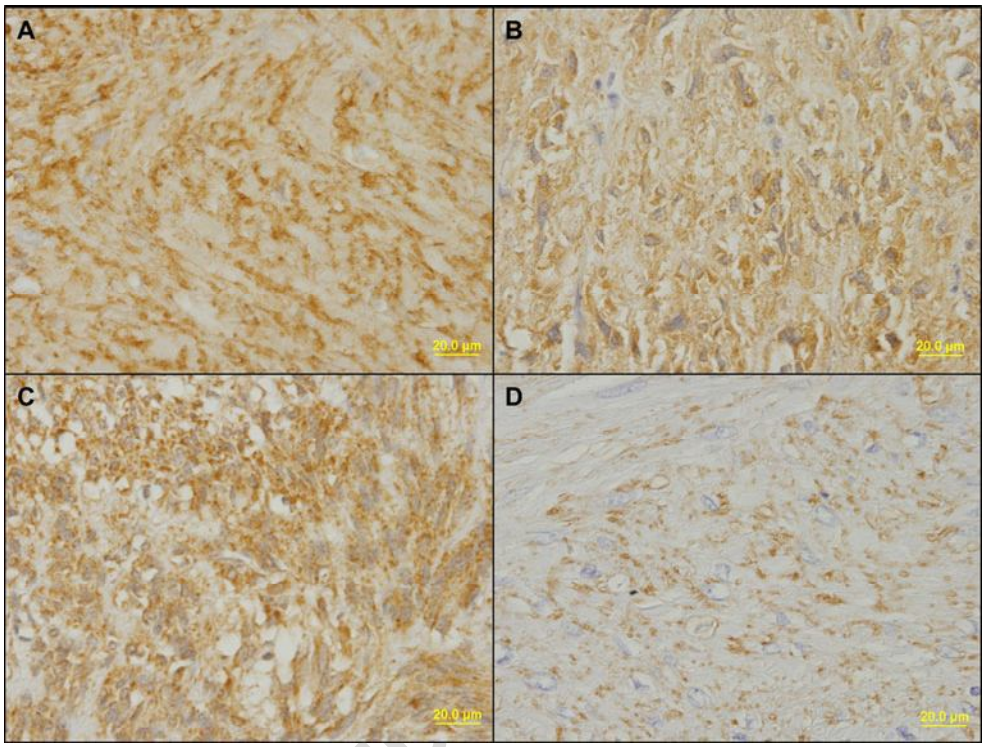

Fig. 1 Immunohistochemical expression of monocarboxylate transporters (MCTs) and CD147 in gastrointestinal stromal tumors (GISTs). MCT1 (**a**), MCT2 (**b**) and MCT4 (**c**) were strongly and extensively observed in the cytoplasm of GIST cells while CD147 (**d**) presented a more focal expression

observed between the genetic status of the cases and expression of MCTs.

Concerning the clinical-pathological data (Table 2), we observed that tumors with absence of necrosis showed higher likelihood of being positive for MCT1, than did those presenting necrosis (p=0.042). Additionally, all patients without locoregional relapse were positive for MCT4, while only 62.5% (5/8) cases with locoregional relapse presented MCT4 positive expression (p=0.014). Importantly, CD147 expression was associated with a progressive increase in Fletcher's Risk of Malignancy (p=0.020). Co-expression of MCT1 or MCT4 with CD147 did not reveal any additional associations with the clinical-pathological data.

Overall survival analysis showed that patients had a median survival rate of 49 months, ranging from 1 to 180 months. Although no associations were observed for each protein individually, we found that patients with tumors co-expressing MCT1and CD147 have a lower overall survival than the other group of patients (81 versus 158 months, respectively, p=0.037, Fig. 2).

t1.1 **Table 1** Association between GIST molecular status and MCT and CD147 expression

t1.2		n	MCT1 positive (%)	p	MCT4 positive [a] (%)	p	CD147 positive (%)	p
t1.3	Mutational status			0.528		0.298		0.039
t1.4	*KIT* mutated	44	38 (86.4)		38 (86.4)		30 (68.2)	
t1.5	*PDGFRA* mutated	7	7 (100.0)		7 (100.0)		3 (42.9)	
t1.6	*KIT/PDGFRA/BRAF* wild-type	10	9 (90.0)		8 (100.0)		3 (30.0)	
t1.7	Mutation location			0.689		0.192		0.137
t1.8	*KIT* exon 9	6	5 (83.3)		6 (100.0)		4 (66.7)	
t1.9	*KIT* exon 11	37	32 (86.5)		32 (86.5)		26 (70.3)	
t1.10	*KIT* exon 17	2	2 (100.0)		1 (50.0)		0 (0.0)	
t1.11	*PDGFRA* exon 18	7	7 (100.0)		7 (100.0)		3 (42.9)	
t1.12	Mutation Type			0.611		0.193		0.356
t1.13	Insertion and deletion	24	21 (87.5)		19 (79.1)		15 (62.5)	
t1.14	Substitution and duplication	21	20 (95.2)		20 (95.2)		16 (76.2)	

[a] 2 missing cases

t2.1 **Table 2** Association between GIST clinical-pathological parameters and MCT and CD147 expression

t2.2		n	MCT1 positive (%)	*p*	MCT4 positive [a] (%)	*p*	CD147 positive (%)	*p*
t2.3	Ascites			0.209		1.000		0.134
t2.4	Absence	42	39 (92.9)		35 (87.5)		25 (59.5)	
t2.5	Presence	9	7 (77.8)		8 (88.9)		8 (88.9)	
t2.6	Fletcher Risk of Malignancy			0.643		0.204		0.020
t2.7	Very Low/Low	14	12 (85.7)		11 (84.6)		4 (28.6)	
t2.8	Moderate	19	16 (84.3)		19 (100.0)		11 (57.9)	
t2.9	High	27	25 (92.6)		23 (85.2)		20 (74.1)	
t2.10	Locoregional relapse [b]			0.583		0.014		0.689
t2.11	Absence	23	20 (87.0)		22 (100.0)		14 (60.9)	
t2.12	Presence	8	6 (75.0)		5 (62.5)		4 (50.0)	
t2.13	Cancer Persistence			0.143		0.665		0.217
t2.14	No	31	26 (83.9)		27 (90.0)		18 (58.1)	
t2.15	Yes	20	20 (100.0)		16 (84.2)		15 (75.0)	
t2.16	Metastasis			1.000		0.077		0.603
t2.17	Absence	28	25 (98.3)		26 (96.3)		19 (67.9)	
t2.18	Presence	23	21 (91.3)		17 (77.3)		14 (60.9)	
t2.19	Tumor size (cm)			1.000		0.325		0.164
t2.20	≤10	41	36 (87.8)		37 (94.9)		21 (51.2)	
t2.21	>10	20	18 (90.0)		17 (85.0)		14 (70.0)	
t2.22	Mitotic index [c]			0.319		1.000		0.538
t2.23	≤5	43	38 (88.4)		38 (90.5)		26 (60.5)	
t2.24	>5	14	14 (100.0)		13 (92.9)		10 (71.4)	
t2.25	Necrosis			0.042		1.000		0.740
t2.26	Absence	40	38 (95.0)		35 (89.7)		23 (57.5)	
t2.27	Presence	21	16 (76.2)		18 (90.0)		13 (61.9)	

[a] 2 missing cases

[b] cases with no persistent disease were also analyzed

[c] per 50 fields of high magnification

Discussion

Cancer research has been traditionally focused on the genetic and epigenetic alterations occurring in tumor development. More recently, greater attention has been given to other components, such as the microenvironment and tumor energetics, as demonstrated by the emergence of a "new" hallmark of cancer—reprogramming energy metabolism (Hanahan & Weinberg 2011), emphasizing the importance of a broader analysis of cancer features.

Cancer metabolism is also gaining relevance in GISTs management. Underlying this phenomenon is the evidence provided by the relationship of glucose uptake, as measured by FDG-PET, with detection of primary GISTs and disease recurrence, pathological risk category (Otomi et al. 2010), as well as patient response to imatinib treatment (Demetri et al. 2002; Cullinane et al. 2005; Holdsworth et al. 2007). In fact, FDG-PET is currently used to evaluate the efficacy of imatinib in GIST patients (Demetri et al. 2002) as well as in preclinical and clinical studies with new inhibitors for GISTs treatment (Pantaleo et al. 2010; Revheim et al. 2010). In chronic myelogenous leukemia (CML), imatinib-sensitive cells showed a decrease in both glucose uptake and lactate production as well as an increase in oxidative metabolism after imatinib treatment, while imatinib-resistant cells maintained the high levels of glucose uptake and lactate production characteristic of CML cells (Kominsky et al. 2009). Accordingly, in an in vivo model based on activating *KIT* mutations in GISTs, a decrease in glucose uptake, measured by FDG-PET, along with a decrease in the glucose transporter GLUT1 expression was observed after imatinib treatment in imatinib-responsive tumors. Therefore, imatinib may also act as a metabolic modulator, by depriving transformed cells from their key substrate, thus contributing to its cytotoxicity (Cullinane et al. 2005). Thus, GISTs with higher glycolytic rates may benefit more from imatinib treatment as well as other kinase inhibitors. As a result, metabolic characterization of GISTs prior and soon after treatment, may have predictive value and be used as an

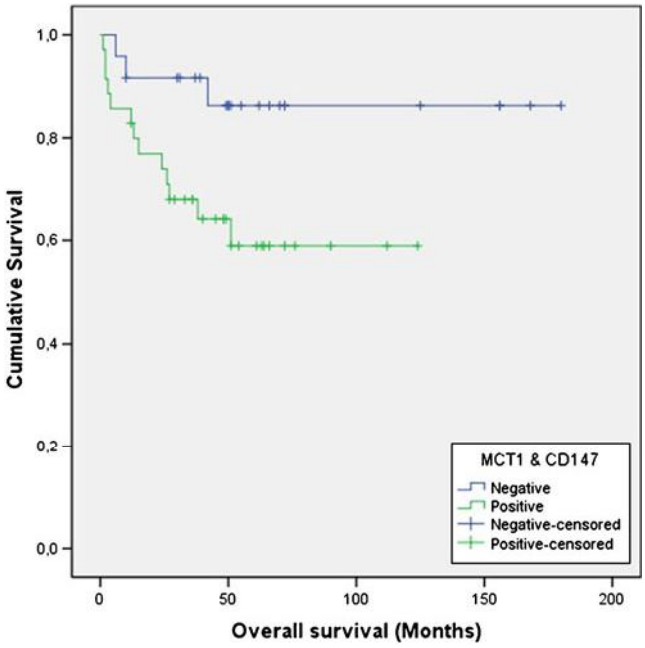

Fig. 2 Overall survival curve regarding MCT1 and CD147 co-expression in GIST patients. Patients with positive tumors for MCT1 and CD147 co-expression show shorter disease-free survival (*green line*) than patients without MCT1 and CD147 co-expression (*blue line*) (p=0.037)

early indicator of response. In this context, MCTs arise as relevant proteins involved in cancer metabolism that should be included in the metabolic characterization of tumors, as lactate transporters and pH regulators.

Evidence for the association between genetic background and tumor metabolism has been appearing in the last few years. It was recently described that in colorectal cancer cell lines, *GLUT1* was consistently up-regulated in cells with *KRAS* or *BRAF* mutations. Conversely, glucose-deprived colorectal cancer cells acquired *KRAS* mutations not present in the parent cells (Yun et al. 2009). *BRAF* V600E mutation was also shown to increase the expression of the metabolic regulator hypoxia-inducible factor-1alpha (HIF-α), at both mRNA and protein levels, in different cancer types including melanoma, colon and thyroid cancer (Kumar et al. 2007; Zerilli et al. 2010; Kikuchi et al. 2009). In the present study, no associations were found between oncogene mutations and the expression of MCTs. However, we found CD147 to be more frequently expressed in *KIT* mutated cases as compared to *PDGFRA* mutated and wild type cases. To the best of our knowledge, this is the first study showing this association and more studies are warranted to confirm and uncover the molecular events underlying the possible regulation of CD147 by mutated *KIT*.

In the present study, we intended to addressed this issue in a series of molecularly (*KIT*, *PDGFRA* and *BRAF*) well characterized GISTs. We report expression of both MCT1 and MCT4 in around 90% of GISTs, which is in accordance with the high glycolytic metabolism characteristic of this type of tumors.

MCT activity largely depends on the location within the cell. In the cancer context, MCT isoforms 1 and 4 should be expressed at the plasma membrane for proper efflux of the accumulating lactate resultant from the high glycolytic rates. However, in the present study, MCT or CD147 expression in the plasma membrane was not clear. In fact, GISTs did not display clear plasma membrane staining as usually observed in carcinomas, probably due to the different cell organization of sarcomas, when comparing to epithelial malignancies, which make positive plasma membrane reactions unclear under microscopic observation.

Although plasma membrane expression was not observed, some significant correlations were obtained with the clinical-pathological data. MCT1 expression was associated with absence of tumor necrosis. However, one should be careful when considering this association with tumor necrosis, since this was evaluated independently from the analysis of immunohistochemical expression of the proteins herein studied. Therefore, this association does not mean that MCT1 is more frequently present in areas without necrosis, which goes against a previous study in breast cancer, showing MCT1 expression in peri-necrotic areas (Pinheiro et al. 2011). Also, MCT4 was unexpectedly associated with less locoregional relapse. As MCT4 contributes to extracellular acidification and lactate accumulation, which in turn are associated with invasion and metastasis (Rofstad et al. 2006; Walenta et al. 2000; Brizel et al. 2001), an association of MCT4 with an increase in locoregional relapse was expected, and not the contrary. Additional studies are warranted to confirm the value of this association. Finally, in accordance to its widely described poor prognostic value, CD147 was associated with high Fletcher Risk of Malignancy.

Importantly, we showed that co-expression of MCT1 and its chaperone CD147 (EMMPRIN) is associated with poor patient survival. Although MCTs have been previously associated with poor prognosis (Pinheiro et al. in press; Fang et al. 2006; Pinheiro et al. 2009b), this is the first study showing an association of MCT1 with lower survival, reinforcing its role in cancer development and/or aggressiveness, as a player in the metabolic adaptations involved in carcinogenesis and progression towards malignancy. This result has clinical relevance as it suggests that GISTs may benefit from novel therapeutic approaches targeting MCT1 and CD147 (Kennedy & Dewhirst 2010).

Conclusions

This study represents the first characterization of MCT protein expression in GISTs. We show that MCTs are highly

expressed in GISTs and that CD147 expression is associated with high Fletcher Risk of Malignancy. Importantly, co-expression of MCT1 with CD147 is associated with lower patient survival. Further studies evaluating the association of MCT expression with other metabolic regulators, such as GLUT1, and assessing the expression of these metabolic markers before and after imatinib treatment, would be of great importance to further clarify the interdependence between imatinib treatment and metabolic response. Altogether, the results herein reported enhance the comprehension of particular aspects of the biological behavior of gastrointestinal stromal tumors (GISTs), namely MCTs, which are important contributors to the metabolic phenotype of cancer cells.

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Conflict of interest statement None declared.

Authors' contributions FB, RMR, ATO and AL were responsible for the study concept and design. CP, ATO, FB and RMR were responsible for study supervision, manuscript drafting and critical revision. MJB, CS, ALC, DM, TBS and VLV were responsible for clinical pathological collection. CP, ATO, OM, CS, SSS and TBS performed the immunohistochemical reactions, data analysis and interpretation and participated in the drafting of the manuscript. CS and AL evaluated the immunohistochemical reactions.
All the authors read and approved the final manuscript.

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