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Monocarboxylate transporter 4 as a prognostic biomarker in patients with colorectal cancer and liver metastases

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ABSTRACT

Objective: This study aims to validate the prognostic significance of the expression of Monocarboxylate Transporter 4 (MCT4) in patients with colorectal liver metastases (CRLM). This study investigated the correlation between MCT4 expression in stromal and tumor cells of colorectal liver metastases (CRLM) with disease-free (DFS) and overall survival (OS) in liver-only colorectal metastases treated with liver resection following neoadjuvant chemotherapy.

Methods: This is a retrospective study of 107 patients with colorectal liver metastases. MCT4 expression in both stromal and tumor cells was studied by immunohistochemistry. The staining was scored semiquantitatively as weak or strong. DFS and OS were calculated using both Kaplan–Meier and multivariate Cox-regression methods

Results: Specimens from 57 patients (53.27%) showed weak levels of stromal MCT4 staining, whereas 50 patients (46.73%) showed strong levels of MCT4 staining. From the statistical analysis, strong stromal MCT4 expression was associated with decreased DFS (HR 1.79; 95% CI, 1.12–2.85; $P = 0.014$) and OS (HR 3.81 95% CI, 1.88–7.72; $P < 0.001$) in univariate analysis. This finding remained significant in multivariate analysis for both DFS and OS (HR 1.95; 95% CI, 1.19–3.17; $P = 0.007$, and HR 4.38; 95% CI, 2.15–8.92; $P < 0.001$ respectively). Tumeur MCT4 expression was not associated with DFS and OS. Five-years DFS and OS rates were 43% and 78% respectively in patients with weak and 15% and 37% respectively in patients with strong stromal MCT4 expression.

Conclusion: Our results indicated that strong expression of stromal MCT4 in CRLM was associated with poor prognosis in patients who undergo liver resection for liver-only colorectal metastases. This finding could be furthermore validated in independent studies and MCT4 could be used as a new biomarker in CRLM and creates the possibility of new studies in targeted therapies.

1. Introduction

Globally, more than one million people are diagnosed with colorectal cancer every year resulting in about 715,000 deaths as of 2010 which is significantly higher compared to 490,000 deaths in 1990 [1]. Since 2012, it is the second most common cause of cancer in women (9.2% of diagnoses) and the third most common in men (10.0% of diagnoses) [2]. Its prevalence is higher in developed than developing countries. Current therapy is a combination of surgery and chemotherapy. Recent advances in chemotherapy have improved overall survival of patients with colorectal cancer, especially the subgroup with metastases, but overall survival still remains relatively low [3]. Patients with resectable colorectal cancer liver metastases have five-year overall survival of 35–58%. However, these patients have high recurrence rate and only about 15% will be disease free ten years after liver resection [4]. Identifying prognostic factors of recurrence could help better therapy planning. In addition, research for new therapies is evolving and elective molecules that could be targeted are a promising area of investigation [5].

To date, a number of prognostic factors such as grade (tumor differentiation), N-status, large bowel obstruction, operation, primary tumor resection, location, number and size of liver lesions, extrahepatic transfer, preoperative CEA level and chemotherapy have been evaluated [6].

Monocarboxylate transporter 4 (MCT4), also known as solute carrier family 16a member, is a membrane transporting protein that

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in humans are encoded by the SLC16A3gene [7]. Northern and Western blotting and EST database analyses showed MCT4 to be widely expressed in glycolytic tissues such as white skeletal muscle fibers, astrocytes, white blood cells, chondrocytes, and some mammalian cell lines. Due to this selective expression, it has been identified that MCT4 serves lactate export from the cytoplasm, produced by glycolysis. The MCT family counts 14 members and only MCT1-MCT4 catalyze the trans membrane proton-coupled transport of lactate [8].

The "reverse Warburg" effect is a two compartment model of cancer cells energy metabolism that was first thorough studied in humans as a concern to breast cancer. According to this model, glycolytic tumor stroma (cancer-associated fibroblasts, CAF's) transfers energy-rich nutrients, such as lactate, to tumor cells, which is the first step in initiating mitochondrial metabolism in these cancer cells [9].

Using the "reverse Warburg" effect, we considered that MCT4 could be a new biomarker for the clinical outcome of patients with colorectal cancer and liver metastases. MCT4 is the major membrane transporter responsible for L-Lactate efflux-export from glycolytic cells and as such is a biomarker of oxidative stress and aerobic glycolysis in the tumor stroma. As proved in breast cancer, MCT4 is overexpressed in stromal fibroblasts, and this overexpression is associated with poor overall survival [10].

We used immunochemistry to investigate the level of expression of MCT4 in the stroma that surrounds cancer cells of colorectal liver metastases. The level of MCT4 expression was then associated with the clinicopathological features of these patients.

2. Materials and methods

2.1. Patient selection and clinical information

We derived our data from two retrospectively surgical databases: 1st Department of Surgery, Laiko General Hospital, University of Athens, Greece and Nicosia General Hospital, University Hospital, Cyprus. Patients who underwent liver resection between January 2001 and December 2012 for CRLM were identified. Eligible patients for the study were patients without extra-hepatic disease, who received neoadjuvant chemotherapy, had a complete tumor resection, did not deceased because of postoperative complications and finally had adequate tissue samples for the evaluation of MCT4 expression.

Exclusion of extra-hepatic disease was performed preoperatively as routine with chest, abdomen and pelvis computed tomography. Resectability was evaluated from an interdisciplinary board constituted by a surgical expert, an oncologist, and a radiologist. All resections were initiated with a curative intent.

All institutional electronic records were evaluated for each patient and data were collected regarding: a) standard demographics, b) primary colorectal tumor, c) CRLM characteristics, d) preoperative chemotherapy, e) response to preoperative chemotherapy, f) liver resection g) DFS and the OS.

2.2. Immunohistochemical staining

Immunohistochemical staining for MCT4 was performed on 3 μm thick formalin-fixed paraffin sections, using a two-step technique after overnight heating at 37°C and subsequent deparaffinization in xylene and rehydration through graded alcohols. After the quenching of the endogenous peroxidase activity, using methanol hydrogen peroxide solution (0.3% in TBS for 30 min) we proceeded to microwave-mediated antigen retrieval in ethylenediaminetetraacetic acid (EDTA) at pH 9.0 for 10 min. Subsequently, sections were incubated overnight at 4°C with the primary antibodies (MCT4, clone sc-50329, Santa Cruz Biotechnology Inc., USA). A two-step technique (Quanto, Thermo – Fischer Scientific Inc., USA) was used. Diaminobenzidine was used as a chromogen. Finally, sections were counterstained with hematoxylin and mounted. As positive controls, we used placenta (MCT4) sections previously known to be highly immunoreactive for the studied markers. Negative controls had the primary antibody omitted and replaced by nonimmune normal serum from the same species as the primary antibody or TBS.

2.3. Immunohistochemical evaluation

The evaluation of the immunohistochemical staining was performed by a pathologist (G.A.) through light microscopic observation, who was unaware of the clinical data of each patient. The immunoexpression of MCT4 both membranous and cytoplasmic was localized to stromal fibroblasts, tumor cells, endothelial cells of blood vessels, adipocytes, and smooth muscle cells. MCT4, mainly membranous, showed more narrow expression in stromal fibroblasts, tumor cells, and inflammatory cells. Immunoreactivity for MCT4 was estimated in a semiquantitative manner by the evaluation of staining intensity (score 0: no staining, score 1: weak staining, score 2: moderate staining and score 3: strong staining) and the extent of positive tumor cells over the total number of tumor cells (score 0: no staining, score 1: < 25% of tumor stained, score 2:25–50% of tumor stained, score 3: 50–75% of tumor stained, score 4: > 75% of tumor stained). For both stromal and tumor cells, a total (staining intensity plus staining extend) equal or less of 5 was categorized as weak expression, while a total higher than 5 was categorized as the strong expression.

2.4. Statistical analysis

Statistical analyses were performed using the Statistical Package for Social Sciences software (SPSS), version 17.0. The primary and secondary endpoints of the study were DFS and OS respectively. DFS was calculated from the date of hepatectomy to the date of disease recurrence and was censored at the last follow-up or at the time of death if the patients remained tumor free at that time. OS was calculated from the time of hepatectomy to the date of cancer-related death and was censored at last follow-up or at the time of unrelated to cancer death. Chi-square test was used for calculating the association between patients' and tumor's categorical characteristics and stromal and tumor cells MCT4 expression. The impact of these features on DFS and OS was analyzed using the Kaplan–Meier method. Survival outcomes between groups were compared with the log-rank test. A P value of less than 0.05 was considered statistically significant. Factors that were associated with the DFS or the OS ($P > 0.1$) in univariate analysis were used for the performance of the multivariate Cox-regression analysis.

3. Results

A total of 107 patients were enrolled. The demographic characteristics of the patients and the characteristics of CRLM at diagnosis are shown in Table 1. The stromal MCT4 expression was strong in 50 patients (46.7%). Immunochemistry staining is shown in Images 1 and 2. As demonstrated in Table 1, there was no statistically significant difference in patient's and CRLM's characteristics between patients with weak and strong stromal MCT4 expression. The median follow-up period was 39 months (2 to 102 months). During the follow-up period, 72 patients (67.3%) developed tumor recurrence and 38 (35.5%) patients died due to progressive disease. Within the group of patients with strong stromal MCT4 expression of the recurrence rate was much higher, as 40 out of 50 (80%) patients developed tumor recurrence. The corresponding rate for the group of patients with weak stromal MCT4 expression was 56.1% (80% vs