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Hodgkin lymphoma: A complex metabolic ecosystem with glycolytic reprogramming of the tumor microenvironment

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ABSTRACT

Background: Twenty percent of patients with classical Hodgkin Lymphoma (cHL) have aggressive disease defined as relapsed or refractory disease to initial therapy. At present we cannot identify these patients pre-treatment. The microenvironment is very important in cHL because non-cancer cells constitute the majority of the cells in these tumors. Non-cancer intra-tumoral cells, such as tumor-associated macrophages (TAMs) have been shown to promote tumor growth in cHL via crosstalk with the cancer cells. Metabolic heterogeneity is defined as high mitochondrial metabolism in some tumor cells and glycolysis in others. We hypothesized that there are metabolic differences between cancer cells and non-cancer tumor cells, such as TAMs and tumor-infiltrating lymphocytes in cHL and that greater metabolic differences between cancer cells and TAMs are associated with poor outcomes.

Methods: A case-control study was conducted with 22 tissue samples of cHL at diagnosis from a single institution. The case samples were from 11 patients with aggressive cHL who had relapsed after standard treatment with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) or were refractory to this treatment. The control samples were from 11 patients with cHL who achieved a remission and never relapsed after ABVD. Reactive non-cancerous lymph nodes from four subjects served as additional controls. Samples were stained by immunohistochemistry for three metabolic markers: translocase of the outer mitochondrial membrane 20 (TOMM20), monocarboxylate transporter 1 (MCT1), and monocarboxylate transporter 4 (MCT4). TOMM20 is a marker of mitochondrial oxidative phosphorylation (OXPHOS) metabolism. Monocarboxylate transporter 1 (MCT1) is the main importer of lactate into cells and is a marker of OXPHOS. Monocarboxylate transporter 4 (MCT4) is the main lactate exporter out of cells and is a marker of glycolysis. The immunoreactivity for TOMM20, MCT1, and MCT4 was scored based on staining intensity and percentage of positive cells, as follows: 0 for no detectable staining in $>$ 50% of cells; 1+ for faint to moderate staining in $>$ 50% of cells, and 2+ for high or strong staining in $>$ 50% of cells.

Results: TOMM20, MCT1, and MCT4 expression was significantly different in Hodgkin and Reed Sternberg (HRS) cells, which are the cancerous cells in cHL compared with TAMs and tumor-associated lymphocytes. HRS have high expression of TOMM20 and MCT1, while TAMs have absent expression of TOMM20 and MCT1 in all but two cases. Tumor-infiltrating lymphocytes have low TOMM20 expression and absent MCT1 expression. Conversely, high MCT4 expression was found in TAMs, but absent in HRS

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cells in all but one case. Tumor-infiltrating lymphocytes had absent MCT4 expression. Reactive lymph nodes in contrast to cHL tumors had low TOMM20, MCT1, and MCT4 expression in lymphocytes and macrophages. High TOMM20 and MCT1 expression in cancer cells with high MCT4 expression in TAMs is a signature of high metabolic heterogeneity between cancer cells and the tumor microenvironment. A high metabolic heterogeneity signature was associated with relapsed or refractory cHL with a hazard ratio of 5.87 (1.16–29.71; two-sided $P < .05$) compared with the low metabolic heterogeneity signature. *Conclusion:* Aggressive cHL exhibits features of metabolic heterogeneity with high mitochondrial metabolism in cancer cells and high glycolysis in TAMs, which is not seen in reactive lymph nodes. Future studies will need to confirm the value of these markers as prognostic and predictive biomarkers in clinical practice. Treatment intensity may be tailored in the future to the metabolic profile of the tumor microenvironment and drugs that target metabolic heterogeneity may be valuable in this disease.

Introduction

It is estimated that over 9,000 new cases of classical Hodgkin lymphoma (cHL) have been diagnosed in the United States in 2016 with over 1,100 deaths, the majority of whom were adolescents and young adults who had relapsed or refractory disease [1]. The most commonly used treatment for cHL in the United States is combination therapy with adriamycin, bleomycin, vinblastine, and dacarbazine (ABVD) with or without subsequent radiation therapy, and approximately 80% of patients are cured with this approach [2]. However, 20% of patients have aggressive cHL, which is defined as relapsed disease or disease that is refractory to initial therapy [2]. In addition, the toxicity of treatment is significant, with high rates of heart and lung disease, secondary malignancies, and compromised fertility, which not only impact quality of life but increase mortality [3]. In fact, patients with cHL who are cured of their lymphoma will die most commonly from complications of treatment [4].

Tools at diagnosis to accurately predict who will relapse or have refractory cHL are imprecise and are not used widely in clinical practice to reduce therapy or use alternative treatments [5]. Also, the majority of patients with high-risk features are cured with their initial therapy [6]. In summary, cHL is an aggressive cancer where we need better predictive biomarkers for relapsed or refractory disease. This should allow us to design rational clinical trials in cHL that provide less intensive curative treatment for the majority of subjects, reducing toxicity, and providing alternative treatments for those patients with high-risk disease to improve long-term outcomes.

The Hodgkin and Reed Sternberg (HRS) cell is the cancer cell in cHL and its origin is a germinal center or post-germinal center B cell [7,8]. Specifically, this large cancer cell is called the Reed Sternberg cell if binucleated or the Hodgkin cell if mononucleated. Interestingly, non-cancerous cells far outnumber cancer cells in cHL tumors and $< 1\%$ of the tumor cells are HRS on average [9,10]. The importance of non-cancer cells in these tumors is highlighted by the fact that the classification of cHL is based on the tumor microenvironment (TME) or non-cancerous cells within the tumor [8]. The four morphologic subtypes of cHL are nodular sclerosis (NS), mixed cellularity (MC), lymphocyte rich, and lymphocyte depleted [7]. The TME in cHL consists of many different cell types, such as TAMs, reactive lymphocytes (RLs), fibroblasts, eosinophils, mast cells, and plasma cells. HRS cells recruit non-cancer cells to the tumor and there is substantial cross-talk between these cells to favor the growth of the cancer cells, allowing proliferation and resistance to cell death [8,9,11[. The mediators of the crosstalk between TME cells and HRS include cytokines, chemokines, immune checkpoint receptors, and the extracellular matrix [8,9,11,12]. Markers of crosstalk between the TME and HRS have been studied in cHL, and the number of macrophages in the tumor is a predictive biomarker of response to therapy [9]. Predictive biomarkers, attempting to reflect the underlying biology of cHL have also been studied. These include circulating cytokines, chemokines, and soluble receptors such as CD30 [13–17]. Analyses of the tumor itself by protein expression or gene expression profiling have also been performed [18–20]. However, none of the above markers is sufficiently reliable to predict outcomes for patients with cHL [2,21]. We set out to investigate if metabolic markers in cHL can serve to predict outcomes.

Mitochondrial metabolism was studied by measuring expression of translocase of the outer mitochondrial membrane subunit 20 (TOMM20). TOMM20 is a translocase found in the outer mitochondrial membrane, which recognizes and transports into mitochondria cytosolic proteins that are subunits of the oxidative phosphorylation (OXPHOS) machinery [22]. The majority of mitochondrial OXPHOS subunits are nuclearly encoded and TOMM20 provides these subunits to the mitochondria [23]. TOMM20 expression is directly related to OXPHOS as measured by oxygen consumption rates and its expression has been shown to be associated with complex IV activity [24–28]. Cytochrome C Oxidase (COX) is the terminal enzyme in the mitochondrial electron transport chain required for OXPHOS to generate ATP [29]. Low COX activity is a marker of mitochondrial dysfunction and is the gold standard to diagnose human myopathies and neurologic diseases because of mitochondrial dysfunction [30–32].

Monocarboxylate transporters are a family of membrane proteins (MCT) that demonstrate proton-linked passive symport of lactate and pyruvate into and out of cells [33]. MCT1 is expressed in many cell types and cancer cell lines and is associated with lactate uptake [33,34]. MCT1 is upregulated and expressed most prominently in cells with increased mitochondrial OXPHOS, such as heart and red muscle, suggesting an important role in lactic acid and ketone body oxidation [34,35]. MCT4 is the main transporter of lactate out of cells with some ability to also export ketone bodies out of cells; it acts as a marker of oxidative stress [34,36–38]. The expression of MCT4 is upregulated by HIF-1α, which is the main glycolytic transcription factor induced by oxidative stress and hypoxia [38]. In sum, MCT1 and MCT4 are markers of metabolic crosstalk.

Markers of metabolism may be valuable to predict relapse in cHL. This is because most cHL tumors have high glucose uptake on the basis of ^{18}F-2-deoxy-glucose positron emission tomography (FDG-PET) scans and that loss of FDG-PET avidity in interim FDG-PET scans has been shown to be associated with a low risk of relapse or refractory cHL [6]. Hence, metabolic characteristics at diagnosis may be valuable as predictive biomarkers of response to therapy. Despite the fact that cHL tumors are very metabolically active with high glucose uptake and that non-cancer cells are the majority of cells within a tumor, a rigorous metabolic characterization of these tumor cell populations has not been undertaken. It is widely recognized that tumors generate lactate, which is the end product of glycolysis, yet it is unknown if all cells in a tumor or