Monocarboxylate Transporter 4 and Caveolin-1 Expression in Squamous Cell Carcinoma of the Oral Cavity

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ABSTRACT

Objective:

To determine the significance of caveolin-1 (cav-1) and monocarboxylate transporter 4 (MCT4) in squamous cell carcinoma of the oral cavity. We hypothesized that high stromal MCT4 would predict a worse prognosis and correlate with low stromal Cav-1.

Study Design:

Retrospective chart review from 2005 to 2009. Immunohistochemical staining of one pathology slide per patient was done to detect Cav-1 and MCT4.

Setting:

Academic tertiary care medical center.

Patients:

42 patients with squamous cell carcinoma of the oral cavity that underwent surgical resection without pre-operative chemotherapy or radiotherapy were identified. 21 patients had stage IV disease, 11 patients had stage III, six patients had stage II and four patients had stage I. The average duration of follow-up was 2.5 years.

Main Outcome Measures:

Disease free survival.

Results:

62% (26 /42) of patients had high stromal MCT4 staining (score = 2 or 3). High MCT4 stromal expression correlated with high Cav-1 epithelial expression (p = 0.02). 62% (26/42) of patients had low epithelial MCT4 staining (score = 1). Low MCT4 epithelial expression was highly correlated with low Cav-1 stromal expression (p = 0.0035). High MCT4 stromal expression was found to correlate significantly with two or more positive lymph nodes (p = 0.03). A statistically significant increase in disease free survival was identified with low epithelial MCT4 staining (score = 1). 83% (35/42) of patients had high epithelial Cav-1 staining (score = 2).

Conclusion:

In patients with high epithelial MCT4 expression, disease free survival was significantly reduced. High stromal expression of MCT4 correlated with two or more positive lymph nodes in patients.

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INTRODUCTION

Caveolin-1 (Cav-1) is a cell surface protein that is a major structural component of caveolae, vesicles of the plasma membrane integral to a variety of signal transduction molecules and transport functions. Caveolin-1 is expressed in a variety of cell types, with abundance found in smooth muscle, adipocytes, endothelial cells, fibroblasts and type I pneumocytes, while central nervous system neurons and lymphocytes are devoid of caveolae (14). Research has shown Cav-1 to act as a scaffolding protein by directly influencing a multitude of signaling cascades including Src-family tyrosine kinases and the Ras family (4,5,7). This ability to modulate signaling has produced significant evidence to suggest that overexpression or downregulation of Cav-1 and caveolae may have important roles in the process of cell transformation, tumor suppression and tumor formation (6,7).

Research has shown Cav-1 to be a tumor suppressor protein that is targeted and down-regulated by activated oncogenes, resulting in suppression of Cav-1; while overexpression or upregulation of Cav-1 results in inhibition of oncogenes and transformation suppression (6,7,8). Loss of stromal Cav-1 in breast cancer patients is a known critical negative prognosticator (3). This loss of stromal Cav-1 is thought to allow infiltration of inflammatory cells, which drives autophagy in the tumor stroma, thus creating an aggressive tumor microenvironment and promoting the growth of cancer cells by providing sufficient nutrients. In addition, the loss of stromal Cav-1 has been shown to result in fibroblasts that trend toward autophagy and aerobic glycolysis secondary to increased oxidative stress, contributing to a nurturing tumor microenvironment. An important marker of this oxidative stress is monocarboxylate transporter (MCT) 4 (13).

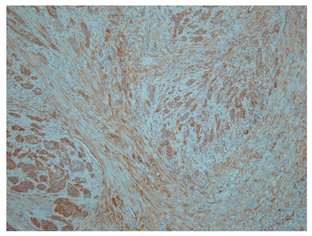
Monocarboxylate transporter 4 is a transport protein believed to play an important role in lactate efflux from glycolytic cells, such as muscle fibers and astrocytes. In recent years, MCT4 expression has been studied in concert with Cav-1 expression in cancer cell lines. MCT4 was identified as a key protein in the cell's response to oxidative stress, which Cav-1 has been shown to up-regulate (12). Recent studies have shown that over-expression of MCT4 by stromal fibroblasts adjacent to breast cancer cells protects both the cancer cells and the fibroblasts from cell death (12,13). Stromal provision of lactate to cancer cells is thought to be the mechanism for this increased cell survival, termed the Reverse Warburg Effect (13). High stromal expression of MCT4 paired with low stromal cav-1 has been shown to better predict prognosis than Cav-1 expression alone (13).

Our objective was to evaluate the significance of Cav-1 and MCT4 in squamous cell carcinoma of the oral cavity.

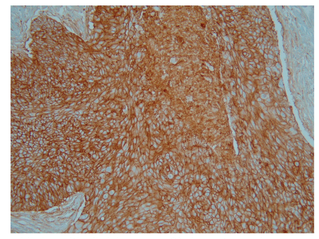
METHODS

A retrospective chart review of patients with oral cavity squamous cell carcinoma at an academic tertiary care medical center was completed. A total of 42 patients met inclusion criteria. Each patient underwent initial surgical resection between 2002 and 2010. Patients had a minimum follow-up of two years and no patients had pre-operative chemotherapy or radiotherapy. Patient age, smoking and drinking status, tumor site, TNM stage, tumor morphology, pre-operative PET results, use of adjuvant chemo or radiation treatment, length of follow up, and recurrence status were obtained.

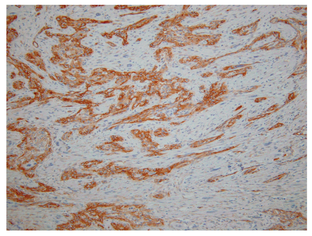
Epithelial and stromal staining patterns were studied and quantified separately from each other. All the tumor on a slide and its dominant staining pattern were considered when determining the percent of immunopositive tumor cells in a sample. The same pathologist scored all samples. A Cav-1 epithelial staining score of 2, 1, or 0 was given if > 50, 10-50, or < 10% of the advancing tumor edge had continuous membranous Cav-1 staining, respectively. A Cav-1 stromal staining score of 2, 1, or 0 was given if > 50, 10-50, or <10% of stromal cells stained positive for Cav-1, respectively (figure 1). An MCT4 epithelial staining score of 3, 2, or 1 was given if >75, 25-75, or <25% of membranous tumor cells stained positive for MCT4, respectively. An MCT4 stromal staining score of 3, 2, or 1 was given if >30, 10-30, or <10% of stromal cells stained positive for MCT4, respectively (figure 1).



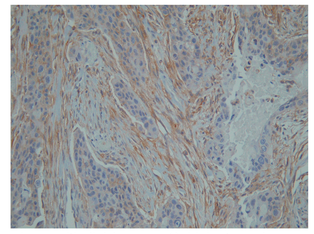
High epithelial (score = 2), high stromal (score = 2) expression Cav-1



High epithelial (score = 3), low stromal (score = 1) expression MCT4



High epithelial (score = 2), low stromal (score = 1) expression Cav-1



Low epithelial (score = 1), high stromal (score = 3) expression MCT4

Figure 1. Cav-1 and MCT4: stromal and epithelial staining in human oral cavity cancer patients. Representative images of patients with different staining patterns. Magnification, 200x

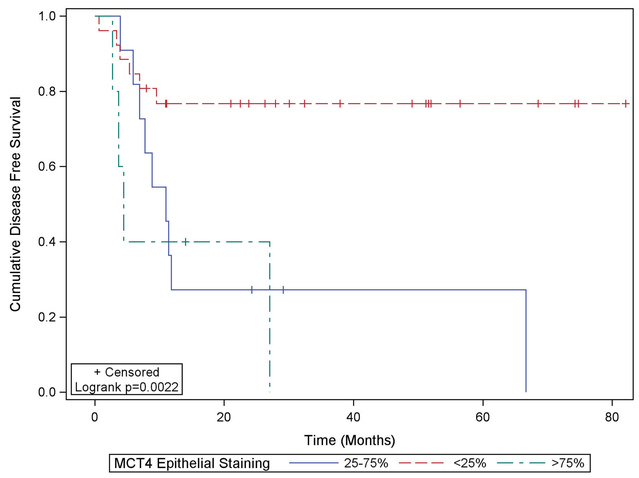


Figure 2. Low MCT4 Epithelial staining, score = 1 (<25%), significantly associated with increased disease free survival (DFS).

RESULTS

Patient Characteristics

42 patients were included, with average age at time of diagnosis of 61 years (range 36 – 83 years old). Males constituted 65% of the patient population. 21 patients had stage IV disease, 11 patients had stage III, six patients had stage II and four patients had stage I. The average duration of follow-up was 2.5 years. Recurrence rate was 38% (16/42).

MCT4 Immunoreactivity

62% (26 /42), of patients had high stromal MCT4 staining, which is a staining score of 2 or 3. High MCT4 stromal expression correlated with high Cav-1 epithelial expression (p = 0.02). 62% (26/42) of patients had low epithelial MCT4 staining (score = 1). Low MCT4 epithelial expression was highly correlated with low Cav-1 stromal expression (p = 0.0035).

MCT4 Immunoreactivity and Clinical Features

High MCT4 stromal expression was found to correlate significantly with two or more positive lymph nodes (p = 0.03). A statistically significant increase in disease free survival was identified with low epithelial MCT4 staining (score = 1). (figure 2)

Cav-1 Immunoreactivity

83% (35/42) of patients had high epithelial Cav-1 staining (score = 2). High epithelial Cav-1 expression correlated with high MCT4 stromal expression (p = 0.02). 38% (16/42) of patients had low Cav-1 stromal staining (score =0 and 1). Low stromal Cav-1 expression correlated with low MCT4 epithelial expression (p = 0.0035).

Cav-1 Immunoreactivity and Clinical features

In patients with less than two positive nodes and negative surgical margins, Cav-1 stromal staining of <50% was found to have a recurrence rate of 30% (7/23), while patients with Cav-1 stromal staining of >50% had no recurrences (0/6) (p=0.18). In patients with two or more positive nodes, 71% (10/14) had < 50% stromal Cav-1 expression.

DISCUSSION

The results of this study suggest that high epithelial expression of MCT4 may predict a poorer prognosis for patients with oral cavity squamous cell carcinoma. In patients with high epithelial MCT4 expression (score=2 or 3), disease free survival was significantly reduced. According to the Warburg Effect, cancer cells rely on a high rate of aerobic glycolysis to obtain sufficient energy in a hypoxic microenvironment for rapid cellular growth (16,17,18). This process creates increased lactate, resulting in greater expression of MCT4, a lactate shuttle. In this study, high epithelial MCT4 expression correlated with a statistically significant reduction in disease free survival, consistent with tumor metabolism and growth as predicted by the Warburg Effect.

High stromal expression of MCT4 was found to correlate with two or more positive lymph nodes in patients, an independent predictor of poor prognosis in head and neck cancer. The Reverse Warburg Effect is one potential explanation as to why high stromal MCT4 could predict a poorer prognosis. This theory states that stromal MCT4 provides lactate to cancer epithelial cells, enabling cancer cells to thrive. Of note, it has been shown that MCT4 expression is upregulated by oxidative stress and Cav-1 is thought to play an integral role in signaling oxidative stress (12). Our study provides additional evidence of a correlation between high stromal MCT4 expression and high epithelial Cav-1 expression.

Patients with Cav-1 stromal staining of <50%, less than two positive nodes and negative surgical margins, were found to have a recurrence rate of 30% (7/23), while patients with Cav-1 stromal staining of >50% had no recurrences (0/6). Although this was not statistically significant (p=0.18), the lack of significance may have been due to small sample size. In addition, low stromal Cav-1 was significantly correlated with low epithelial MCT4 staining (p=0.0035). Previous studies have shown that high stromal MCT4 correlates with a worse prognosis (12,13). Although our results showed a correlation between high stromal MCT4 and two or more positive nodes, high epithelial MCT4 expression was found to independently predict decreased disease free survival. These results might be explained by variation in the cancer cell nutrient source (stroma versus epithelium) between tumor types.

Evidence of correlation between (a) increased stromal MCT4 expression and positive nodal disease, and (b) decreased disease free survival and increased epithelial MCT4 expression, may offer new opportunities to develop more targeted therapeutic strategies for patients with oral cavity cancer, as well as improved ability to address the disease with existing treatment modalities. It has been suggested that cancer cells and the surrounding stroma are dependent on the Warburg Effect or the Reverse Warburg Effect, suggesting an exploitable opportunity for therapy targeted at cancer cell metabolism (18).

CONCLUSIONS

This is the first investigation into the role of stromal Cav-1 and MCT4 expression in oral cavity squamous cell carcinoma. Our research indicates that high epithelial MCT4 expression predicts decreased disease free survival. In patients with less than two positive nodes and negative surgical margins, low stromal Cav-1 may have prognostic significance for an increased risk of recurrence. Further studies may be able to demonstrate statistical significance and additional investigation is warranted.

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