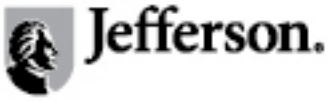

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Tumor Metabolism in the Microenvironment of Nodal Metastasis in Head and Neck Squamous Cell Carcinoma

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TUMOR METABOLISM IN THE MICROENVIRONMENT OF NODAL METASTASIS IN HEAD AND NECK SQUAMOUS CELL CARCINOMA

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ABSTRACT

Introduction: Monocarboxylate transporter 4 (MCT4) is a cell membrane transporter of lactate. MCT4 is a tumor-specific marker of oxidative stress, glycolysis and hypoxia in tumor stromal cells. We investigated HPV positive and negative tumors with regional metastases to cervical lymph nodes (LN) to study how the metastatic tumor cells interact with their microenvironment. By selecting cancers with extracapsular extension (ECE), we intended to evaluate the interaction between metastases and the surrounding extranodal tissue. **Methods**: Clinical data were collected from 24 advanced stage oropharyngeal squamous cell carcinoma (OPSCC) patients with neck LN metastasis. All patients presented with at least N1 disease and had ECE. Sixteen cases were negative for HPV and eight were positive. Ten patients (42%) had ECE < 1 mm, and 14 (58%) had ECE > than 1 mm. The extent of ECE was quantified on H&E stains by distance from the edge of capsule. The paraffin-embedded metastatic LN sections were stained with MCT4 and quantification was accomplished using the Aperio Co-localization algorithm. **Results:** High stromal MCT4 expression was strongly associated with the extent of ECE regardless of HPV status (p=0.031). The stromal MCT4 expression in ECE area was significantly higher as opposed to the surrounding extranodal tissue adjacent to intact capsule (p<0.001). We also found a borderline difference in expression of MCT4 in HPV- LN with ECE >1mm vs. <1mm(p=0.06). **Conclusions:** MCT4 is a marker of oxidative stress and higher expression of stromal MCT4 in ECE area is significantly correlated with the extent of ECE. The stromal cells separating nests of cancer cells in ECE area have apparent expression of the MCT4. Together these findings provide new insight into the critical role of stromal MCT4 in nodal metastasis and ECE in OPSCC and it may be useful to develop a novel prognostic marker and new anti-cancer agents.

INTRODUCTION

Nodal metastasis in HNSCC is a strong driver of poor prognosis. Survival is further worsened if lymph node metastases are found to have ECE. The tumor microenvironment clearly plays a critical role in many of the processes involved in tumorigenesis; coupling of metabolism between tumor regions within the microenvironment contributes to tumor metastasis and invasion. The pathophysiologic mechanism of ECE is not fully understood. The process of nodal metastasis and ECE involves a series of complex interactions within the microenvironment of the tumor and lymph node. MCT4 is a transporter of pyruvate and lactate out of cells; its expression is upregulated in the settings of hypoxia and inflammation. This study aims to characterize the metabolic microenvironment of metastatic HNSCC, and especially its role in extracapsular extension in lymph node metastases.

METHODS

After IRB approval, records of 24 consecutive patients treated surgically for oral cavity squamous cell carcinoma and identified extracapsular lymph node extensions were reviewed. Fourteen tumors were HPV-negative and eight were HPV-positive. Clinical data were collected for each patient. Nodal Specimens were immunohistochemically stained for MCT4 antibody (Santa Cruz Biotechnology). Aperio accomplished quantification of immunohistochemistry staining using digital pathology interpretation. Intensity scores were compared against clinical data to identify potential biomarkers for favorable or unfavorable tumor behavior.

RESULTS

Pathologic features are described in Table 1. Hematoxylin and eosin (H&E) staining of lymph nodes with computer-assisted measurement to determine extent of extracapsular extension (ECE). Top: H&E, ECE greater than 1 mm (macro-ECE), Middle: MCT4 staining (Annotated region by Aperio). Bottom: H&E, ECE less than 1 mm (micro-ECE).

Patient demographics and pathologic features

Table 1. Demographics and Pathologic Features		
Demographic	Micro ECS	Macro ECS
Sex	m 6/f 3	m 9/f 4
Mean age (yr)	58.8 (52-69)	59.7 (44-81)
Tobacco Use	8	10
Alcohol Use	7	9
Primary Tumor Site		
Oropharynx		
BOT	1	2
Tonsil	1	1
GT Sulcus	1	1
Oral Cavity		
Oral Tongue	3	2
FOM	3	5
Hard Palate	0	2
Pathologic Features		
Well-moderately diff.	6	12
Poorly diff.	3	1
Prognositc Factors		
HPV (+)	3	5
HPV (-)	6	8
PNI	3	9
LVI	2	11
Positive Surgical Margins	0	0
Adjuvant Treatment		
XRT	9	11
Systemic Therapy	9	11

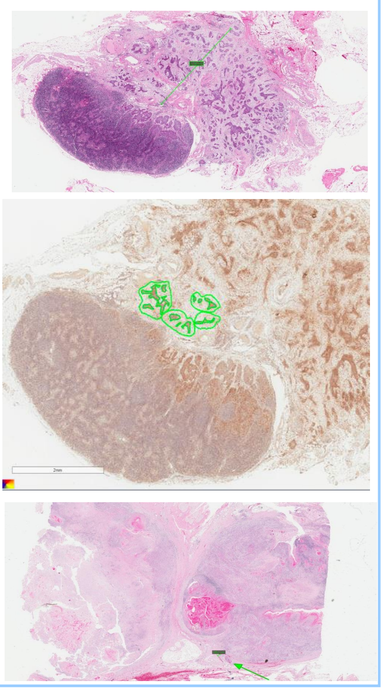

Table 2, HPV Negative vs. Positive tumors; Recurrence and Survival for micro and macro ECE, Figure 2. Kaplan-Meier curve for disease-free survival in micro-ECE patients (red) and macro-ECE patients (blue), p=0.022.

Recurrence and Survival for micro- and macro-ECE

Table 2. Recurrence for Micro and Macro ECE and HPV status.	ECE <1mm	ECE >1mm
Recurrence		
Total (HPV-/HPV+)	2 (2/0)	8 (6/2)
Local	1	6 (4/2)
Regional	1	5 (4/1)
Distant	2	3 (3/0)
Survival		
DFS	20.6 mos.	12.2 mos.
OS	21.7 mos.	14.5 mos.

Table 2

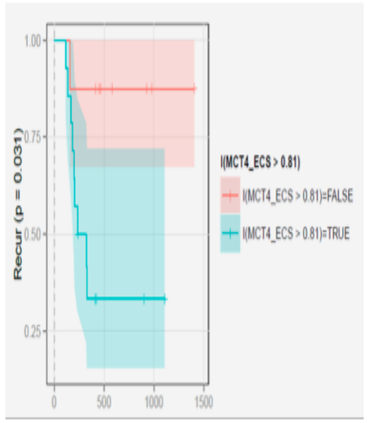

Figure 2

CONCLUSION

Metabolic coupling occurs in HNSCC at sites of cervical metastasis. MCT4 expression was higher in peri-nodal stroma invaded by tumor as compared to those areas away from tumor on digital image analysis (p<0.001). Also, MCT4 stromal staining was significantly higher around nodes with macro-ECE compared to micro-ECE (p<0.001), suggesting a stronger shift toward glycolytic metabolism in the areas with greater ECE. The Micro and Macro ECE groups were compared for disease-free survival (DFS), and the Macro ECE group had a significantly worse prognosis in this study. MCT4 in peri-nodal stromal tissues served as a biomarker for significant extracapsular extension and worsened disease-free survival among patients with regionally metastatic oral and oropharyngeal HNSCC.

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