

Abstract

The aggressive behavior of oral cavity squamous cell carcinoma (OSCC) leads to clinical treatment challenges and poor prognosis. Over the past several decades, despite many innovations in treatment strategies, overall 5-year survival rate is still only approximately 60%. The tumor microenvironment (TME) plays a critical role in altering patient immune responses through interactions between tumor and immune cells and soluble components. Exosomes are vesicles produced by multivesicular bodies in the cellular endosomal pathway and secreted into the extracellular milieu. These extracellular vesicles have a particle diameter of 30-100 nm and are secreted from cancer cells in a higher proportion than from healthy cells. Recent studies have shown their involvement in multiple stages of cancer progression through their ability to circulate and carry tumor molecules that interact with other cells. Exosome interactions with immune components in the TME can manipulate the anti-tumor response, supporting tumorigenesis, angiogenesis, immune evasion as well as development of chemo-resistance, and metastasis. We hypothesize that differential expression of the exosomal marker CD63 correlates with pathological progression, i.e. mild, moderate, severe dysplasia and invasive squamous cell carcinoma, in OSCC patient specimens. Standard IHC staining methods were used to assay the expression of CD63 followed by expression level histoscore by two blinded observers. Exosomal CD63 expression increased along the dysplasia spectrum from normal to carcinoma, indicating that this biomarker is associated with malignant transformation in oral cavity cancer. This study suggests that exosomes may provide an additional target to enhance drug and immunotherapy in oral cancer.

Introduction

Despite considerable success in treating a variety of cancers with immune checkpoint inhibitor therapies, over 80% of patients with recurrent/metastatic oral cavity squamous cell carcinomas (OSCCs) **do not** derive benefit from immunotherapy. In fact, head and neck/oral cavity SCCs are considered among the most immunosuppressive malignancies, and the tumor microenvironment (TME) plays a major role in immune evasion and dampening anti-PD-1/PD-L1 responses.

Recently, the role of small extracellular vesicles in multiple stages of cancer progression has come to the forefront. These exosomes, 30-100 nm vesicles of endosomal pathway origin, carry a variety of unique protein, lipid, and nucleic acid cargoes and are important players in intercellular communication. Cancer cells have been shown to produce many more exosomes than normal cells, which alter intercellular communication, carry immunosuppressive factors and/or ligands such as PD-L1, and can serve as drug target decoys. Exosome interactions with the TME immune component can manipulate the anti-tumor response, supporting tumorigenesis, angiogenesis, immune evasion, and metastasis as well as development of chemo-resistance. Exosomes are implicated in cisplatin resistance for example, which has a significant negative impact on OSCC treatment responses and patient prognosis.

We hypothesize that differential expression of the exosomal marker CD63 correlates with pathological progression, i.e. mild, moderate, severe dysplasia and invasive squamous cell carcinoma, in OSCC patient specimens.

Methods

Samples from patients with histologically confirmed oral potentially malignant disorders and oral cancer who presented to the Department of Head and Neck Surgery, Roswell Park Cancer Institute, New York, USA (2006 to 2013) were investigated. Patients with clinically and histologically normal oral mucosa were used as controls. For consistency, one section from each sample was H&E stained and the samples were assessed by one (or two) pathologist(s) for histological verification. patient samples were categorized as Normal, Mild Dysplasia, Moderate Dysplasia, Severe Dysplasia and Carcinoma. Standard Immunohistochemical staining included de-paraffinization followed by antigen retrieval and immunostaining for primary antibodies against anti CD63 (Abcam USA, Ab 216130, at 1:50 dilution). Antibody detection was with a mouse and rabbit specific HRP/DAB (ABC) secondary antibody detection kit (Abcam, ab64264) following the manufacturers' protocol. Sections were then counterstained with Mayer's hematoxylin and mounted. Slides were scanned (Aperio ScanScope XT 1509, AT2, Leica Biosystems, IL, USA) at 20x magnification, reviewed independently by two observers, and images analyzed using the Aperio Imagescope v.11.2.0.780 (Leica Biosystems, IL, USA). The sum of the percentage and intensity of positively stained tumor cells within the tissue section was used to generate a histoscore (H-score), which ranged from 0 to 300. Negative staining=0; weak staining=1+; moderate staining=2+; strong staining=3+. H-score=(1 × %1 +ve area across the section)+(2 × % 2+ve across the section)+(3 × % 3+ve across the section). Scores from both reviewers were then averaged. Samples with large differences between reviewer scores were re-assessed and re-scored together. The reviewers were blinded to the histopathological diagnosis during the entirety of the scoring. Statistical analyses were carried out in SPSS software and correlation significance assessed with the Kruskal-Wallis Test.

Results

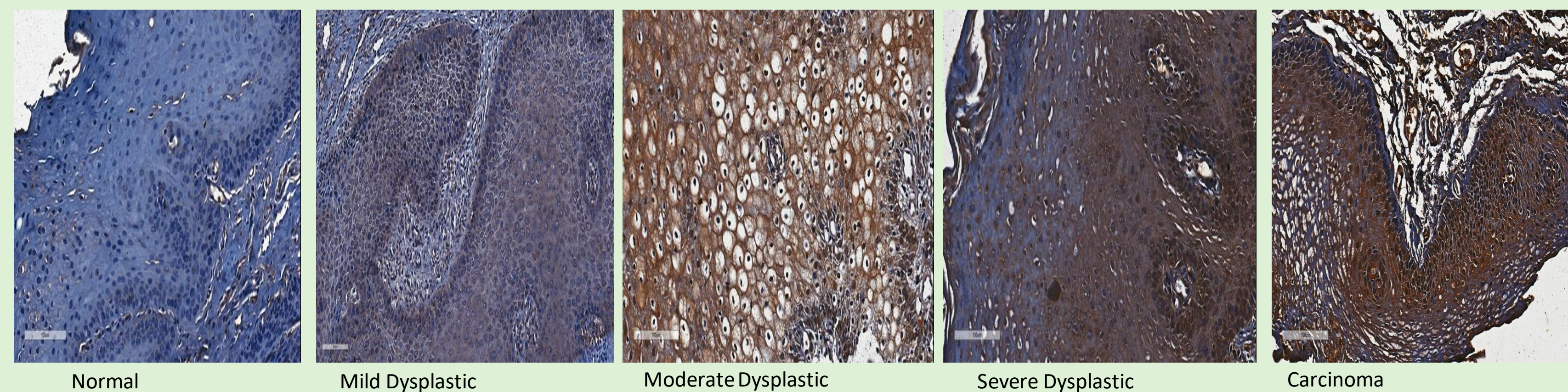


Fig 1: Representative images of CD63 expression(400x) reveals the differential expression of exosomes during oral carcinogenesis. Intensity differences in the brown color represent different levels of exosome expression.

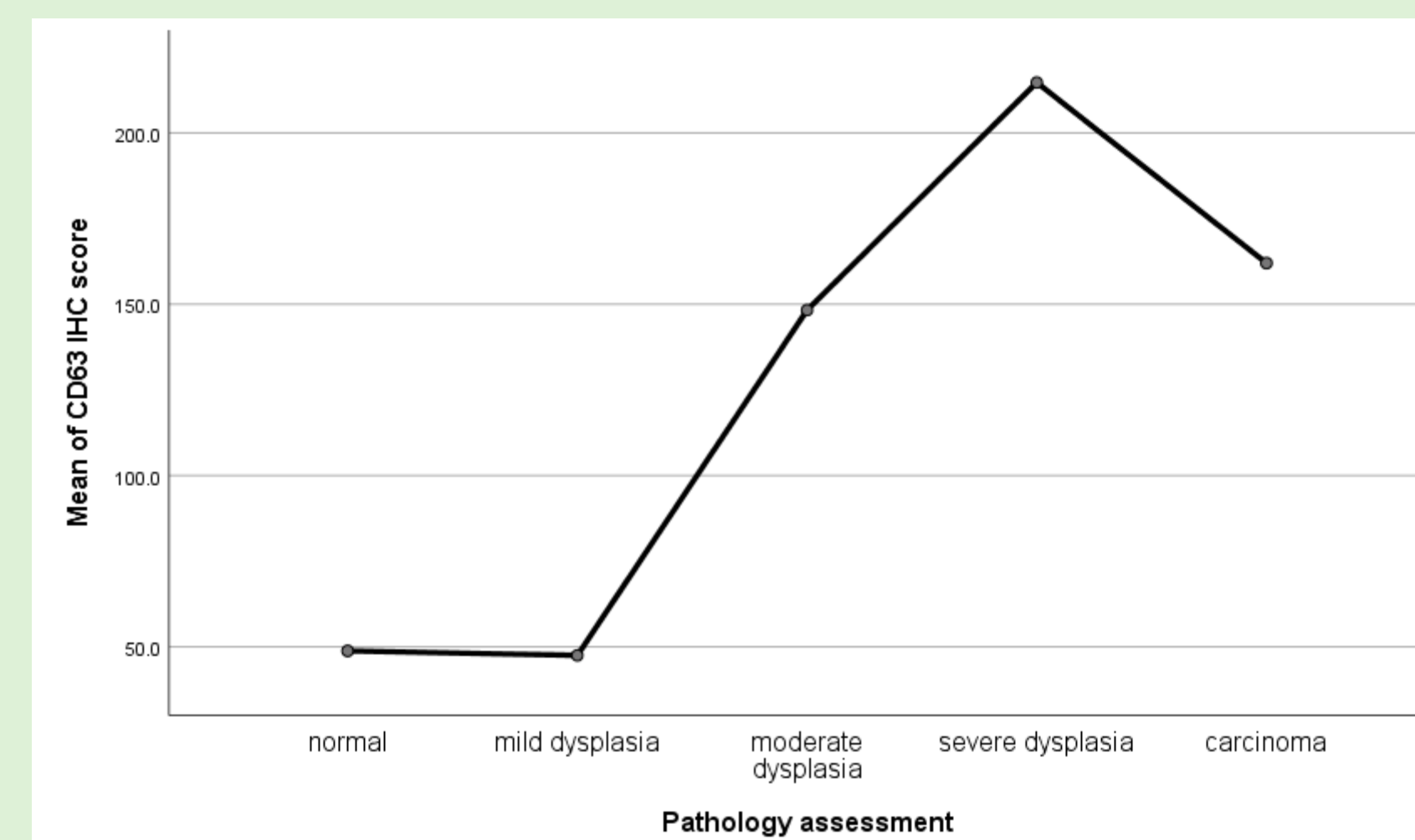


Fig. 2: Mean CD63 IHC score among oral pre-malignant and malignant samples

Results

- The cohort of 252 premalignant and malignant head and neck tissue samples was evaluated for exosomal marker CD63 expression
- Expression of the exosome marker CD63 increased significantly along the dysplasia spectrum and in carcinoma (Figure 1).**
- The mean H-score for CD63 expression was significantly higher in the cancer and severe dysplastic cohorts (214.71±15.31 and 162±16.2 respectively) compared to normal (48.8±5.35), mild dysplastic (47.51±5.8) and moderate dysplastic (148.27±15.85) (**Figure 2**)
- CD63 significantly correlated with disease progression (p<0.001).**

Conclusions

- This study demonstrated that expression of the exosomal marker CD63 increased along the spectrum of dysplasia in oral cavity tissues and correlated significantly with disease progression.
- CD63 expression profile in OPMD and OSCC suggest that tumor cell-associated exosomes may act as a marker for and play an important role in oral cavity cancer progression
- Exosomes therefore may represent a therapeutic target, both as anti-tumor agent delivery vehicle and as direct target for inhibition