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ENDOTHELIN-1 PROMOTES EPITHELIAL MESENCHYMAL TRANSITION IN ORAL SQUAMOUS CELL CARCINOMA

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INTRODUCTION

Oral Squamous cell carcinoma (OSCC) is one of the most common and deadliest malignancies in the head and neck region with the survival rate ranging between 25%-55%. Epithelial-mesenchymal transient (EMT) plays a major role in cancer progression and tissue invasion and is considered to be the key process associated with recurrence, lymph node metastasis and low survival rate in patients with OSCC. Endothelin-1 (ET-1), a potent vasoconstrictor has been shown to promote EMT in various human cancers. However, the role of ET-1 in the progression of OSCC has not been explored, especially in recurrent cases. The current study was done to evaluate and correlate the expression of ET-1 and EMT markers in primary and recurrent cases of OSCC using immunohistochemsitry and Western blot

METHODOLOGY

The study protocol was approved by the Institutional Ethics Committee and written informed consent was obtained from all the participants. Tumor tissues and adjacent clinically normal tissues were taken from 75 patients with primary oral squamous cell carcinoma and 25 patients with recurrent OSCC. The expression of ET-1 and EMT markers, SLUG, TWIST-1, E-cadherin and N-cadherin were analysed using immunohistochemsitry. The tissue samples were incubated with primary anti-mouse monoclonal antibodies against ET-1, SLUG, TWIST-1, E-cadherin and N-cadherin (Dako, USA) and the IHC analysis was performed using standard protocol. The mean immunoreactive score (staining intensity x proportion of positive cells) was calculated and compared between tumor tissue and adjacent normal tissue and also between primary and recurrent cases of OSCC. The immunohistochemical expression of these markers were correlated with pathological grade, lymph node metastasis and AJCC stage of the tumor. Additionally, the expression of ET-1 and TWIST-1 proteins were assessed in human OSCC cell line (SCC180, Sigma, USA) using Western blot analysis. The migration assay was performed using transwell insert and for invasion assay, filters were precoated with 30 microliter matrigel basement membrane matrix (Biosciences, USA) for 30 minutes. After the treatment with ET-1 (0, 10, 30 and 100 nM) for 24 hours, cells were harvested and seeded to Transwell at 1×104 cells/well in serum-free medium and then incubated for 24 hours at 37 °C in 5% CO2. Cells were then fixed in 3.7% formaldehyde for 5 min and stained with 0.05% crystal violet in PBS for 15 min. Cells on the upper side of the filters were removed with cotton-tipped swabs, and the filters were washed with PBS. Cells on the underside of the filters were examined and counted under a microscope.

RESULTS & DISCUSSION

The mean immunoreactive scores of ET-1, SLUG, TWIST-1 and N-cadherin were higher in tumor tissues compared to adjacent normal tissue in patients with OSCC, whereas E-cadherin showed lower expression in OSCC samples (Figure 1). The tissue samples taken from recurrent cases of OSCC had higher expression of both Endothelin-1 and EMT markers than that of the primary OSCC. High grade tumors and those with lymph node metastasis exhibited significantly increased immunohistochemical expression of ET-1, SLUG and TWIST-1 (p=0.01). Western blot analysis showed higher expression of ET-1 and TWIST-1proteins in OSCC cell line (Figure 2). ET-1 enhanced cell migration and invasion of human SCC180 cell line (Figure 3). Following pretreatment of OSCC cells with BQ123 (endothelia antagonist), it was observed that BQ123 inhibited ET-1 induced cell migration (Figure 4).

Figure 1: Immunohistochemical expression of (A) ET-1 (B) TWIST-1 (C) SLUG (D) E-cadherin (E) N-Cadherin in OSCC samples

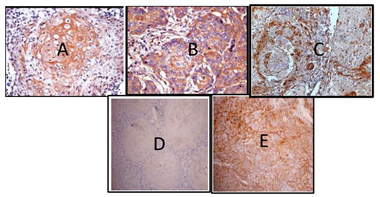


Figure 2: Higher expression of ET-1 (2a) and TWIST-1 (2b) proteins in OSCC cell line

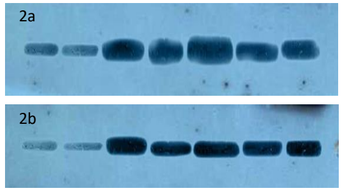


Figure 3: Migration and invasion of OSCC cells were increased by ET-1 (0-100 nM)

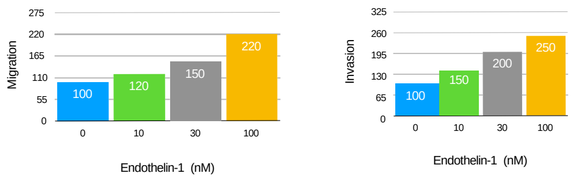
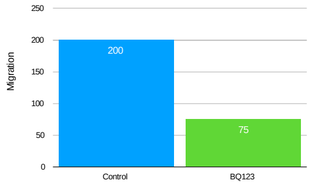


Figure 4: OSCC cells pretreated with BQ123 followed by ET-1 shows decreased migration when compared to control group without ET-1



CONCLUSION

Our study establishes the role of ET-1 in the progression of OSCC and hence, may represent a novel therapeutic target in the treatment of OSCC.

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"The author declares that she has no relevant or material financial interests related to the research described in this poster"