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ORIGINAL ARTICLE

MUC4 regulates cellular senescence in head and neck squamous cell carcinoma through p16/Rb pathway

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The limited effectiveness of therapy for patients with advanced stage head and neck squamous cell carcinoma (HNSCC) or recurrent disease is a reflection of an incomplete understanding of the molecular basis of HNSCC pathogenesis. MUC4, a high molecular weight glycoprotein, is differentially overexpressed in many human cancers and implicated in cancer progression and resistance to several chemotherapies. However, its clinical relevance and the molecular mechanisms through which it mediates HNSCC progression are not well understood. This study revealed a significant upregulation of MUC4 in 78% (68/87) of HNSCC tissues compared with 10% positivity (1/10) in benign samples ($P = 0.006$, odds ratio (95% confidence interval) = 10.74 (2.0–57.56). MUC4 knockdown (KD) in SCC1 and SCC10B HNSCC cell lines resulted in significant inhibition of growth *in vitro* and *in vivo*, increased senescence as indicated by an increase in the number of flat, enlarged and senescence-associated β-galactosidase (SA-β-Gal)-positive cells. Decreased cellular proliferation was associated with G_0/G_1 cell cycle arrest and decrease expression of cell cycle regulatory proteins like cyclin E, cyclin D1 and decrease in BrdU incorporation. Mechanistic studies revealed upregulation of p16, pRb dephosphorylation and its interaction with histone deacetylase 1/2. This resulted in decreased histone acetylation (H3K9) at *cyclin E* promoter leading to its downregulation. Orthotopic implantation of MUC4 KD SCC1 cells into the floor of the mouth in nude mice resulted in the formation of significantly smaller tumors (170 ± 18.30 mg) compared to those (375 ± 17.29 mg) formed by control cells ($P = 0.00007$). In conclusion, our findings showed that MUC4 overexpression has a critical role by regulating proliferation and cellular senescence of HNSCC cells. Downregulation of MUC4 may be a promising therapeutic approach for treating HNSCC patients.

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) is the sixth most common cancer with approximately 650 000 incidences and 350 000 deaths worldwide annually.[1] In the United States, 41 380 new cases and 7850 HNSCC-related deaths were expected in 2013.[2] Despite multiple treatment modalities, including surgery, chemotherapy and/or radiotherapy, the 5-year survival rate has not improved beyond 40–50% in last three decades[3] and can be attributed to the incomplete understanding of the molecular basis of HNSCC pathogenesis. Therefore, unraveling the cellular pathways for improved understanding of HNSCC pathogenesis is urgently needed.

Mucins are a family of heavily O-glycosylated proteins protecting epithelial cell surfaces under normal physiological conditions. Several studies from our lab and others have identified mucins as potential tumor markers and attractive therapeutic targets.[4,5] MUC4, a membrane-bound mucin is expressed in several normal tissues, but its expression is elevated in malignancies of ovaries, thyroid, pancreas and breast.[6] Studies from our lab have shown that ectopic overexpression of MUC4 induces neoplastic transformation of fibroblasts suggesting the oncogenic potential of MUC4.[7] MUC4 activates Src/focal adhesion kinase (FAK) signaling by physical interaction and stabilization of human epidermal growth factor receptor 2,[8,9] thereby promoting survival, invasion and metastasis.[8,9] More recently, we have shown that MUC4 stabilizes N-cadherin expression and promotes epithelial-to-mechenchymal transition in pancreatic cancer cells.[10]

We studied MUC4 expression in human HNSCC tissues and investigated its functional role in HNSCC cell lines. We observed significant upregulation of MUC4 in HNSCC tissues compared with normal tissues. Knockdown (KD) of MUC4 in HNSCC cells reduced proliferation; led to cell cycle arrest and induced cellular senescence by modulating p16/Rb tumor-suppressor pathway. Furthermore, MUC4 KD decreased pRb and histone deacetylase 1/2 (HDAC1/2)-mediated histone acetylation (H3K9 acetylation) at cyclin E promoter resulting in transcriptional silencing of *cyclin E*. This study is the first to propose that MUC4 KD triggers a senescence response by regulating p16, cyclin D1 and cyclin E expression.

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