ABSTRACT

Head and neck squamous cell carcinoma (HNSCC) is the seventh most fatal cancer in the world, accounting for approximately 300,000 deaths per year. It is known that HNSCC is a highly inflammatory cancer, and that prolonged exposure of human neoplasms to inflammatory mediators promotes EMT, tumor growth, invasion, and metastasis. Interleukin-1 beta (IL-1)b is an inflammatory mediator elevated in the HNSCC microenvironment and associated with EMT and tumor growth. We have already shown that IL-1b upregulates the E-cadherin transcriptional repressor Snail. Herein, we report that inflammatory mediators may also upregulate IMP-3 through Snail, thus further defining the cycle by which inflammation promotes tumor progression. Using microarray, Western blot, and RT-PCR data we show that Snail overexpression lines have significantly increased IMP-3 expression, and that IL-1b in the tumor microenvironment significantly upregulates IMP-3 expression in HNSCC cell lines. We also identify NFkB-dependent regulation of IMP-3.

INTRODUCTION

HNSCCs are notably aggressive cancers which cause death primarily due to metastases. Metastasis involves a complex change in epithelial cell behavior wherein the cell locally invades through the epithelial basement membrane, enters blood or lymph vessels, travels through the circulation, exits the vessel, and replicates at a distant organ. Growing evidence suggests such enhanced mobility requires the cell undergo an epithelial-mesenchymal transition. EMT is a normal part of embryonic development. Therefore, it has been hypothesized that in adults the reactivation of genes involved in embryonic EMT underlies the mechanism of cancer metastasis.

Our goal is to uncover the genes involved in the inflammation induced promotion of EMT in HNSCC. Herein, we examine insulin-like growth factor-II mRNA binding protein-1 (IMP-3). IMP-3 is a 580 kDa oncothermal protein upregulated in a wide variety of cancers including oral squamous cell carcinoma. IMP-3 is believed to stimulate cell proliferation and inhibit apoptosis by acting as a translational activator of IGF-II mRNA. Most importantly, IMP-3 expression correlates with significantly worse histopathologic grade, LNM, tumor classification, and clinical stage in oral squamous cell carcinoma, making it a molecule of clinical significance.

MATERIALS & METHODS

Two well-characterized human HNSCC cell lines, Tu686 and Tu212 were utilized as parents in this study. Tu686 and Tu212 Snail overexpressing, Snail shRNA knockdown, and NFkB knockdown cell lines were also used. A microarray was performed. Tu686 and Tu212 were exposed to recombinant human IL-1b (BD Bioscience). Proteins were resolved by SDS PAGE and analyzed by Western blot. Antibodies: anti-Tu212 were exposed to recombinant human IL-1b were used. cDNA was prepared with a kit (Invitrogen) following the manufacturer's instructions (Invitrogen, Carlsbad, CA). The cDNA was prepared with a kit (Invitrogen) according to the manufacturer's instructions. mRNAs levels were quantified by real-time reverse transcriptase polymerase chain reaction (RT-PCR) using the SYBR Green quantitative PCR kit from Bio-Rad in an MyQ Cycler (Bio-Rad, Hercules, CA) following the manufacturer's protocol.

RESULTS

We provide the first report indicating the role of IMP-3 in the inflammation-induced promotion of EMT in HNSCC. We have already reported that IL-1b in the tumor microenvironment upregulates Snail which then downregulates E-cadherin. Our data now suggests that IL-1b also increases IMP-3 expression, and that increased Snail expression upregulates IMP-3. Thus, we conclude that IL-1b can upregulate IMP-3 through Snail. We also document NFkB-dependent regulation of IMP-3. This newly defined pathway has important implications for chemoprevention and novel therapies. Tailoring individual treatment strategies to aggressively treat HNSCC will improve long-term survival.

CONCLUSIONS

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REFERENCES cited

We refer to the original publications for details of the experimental procedures and the results presented.

PROINFLAMMATORY MEDIATORS UPREGULATE IMP-3 IN HNSCC

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