JUN, FOS, & AP1 Are Upregulated in High Nitric Oxide (NO) Exposed Head & Neck Cancer Cells

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ABSTRACT

Hypothesis: Adaptation to Nitric Oxide (NO) by five Human Head & Neck (H&N) cancer cell lines will result in up-regulated JUN, FOS & AP1; genes related to increased metastatic potential.

Objectives: Adaptation to high concentrations of NO causes the H&N tumor cells to demonstrate a more aggressive phenotype in comparison to non-adapted cells, which result in greater metastatic potential. Little is known about the process of how high NO cells acquire a more aggressive phenotype. In order to mimic what is seen clinically, five H&N cell lines were adapted to high concentrations of NO. A gene chip analysis was used to assess genome wide gene expression. JUN, FOS, & AP1 were identified in all five high NO cells lines as up-regulated. The three genes also demonstrated increased cell migration velocities.

Methods: Five human H&N cells lines (SSC-016, SSC-040, SSC-056, SSC-114, and SSC-116) were studied. The cell lines were adapted to high NO by slow exposure to increasing quantities of DETA-NONOate (NO donor). mRNA isolated from both the parent and NO cell lines were tagged with red/green fluorescent markers and used to complete a genome gene chip analysis. Cell migration rates were assessed via scratch assays.

Results: JUN, FOS and AP1 were consistently up-regulated in all the cell lines. Increased migration velocities were demonstrated compared to the parent cell lines.

Conclusions: Adaptation to high NO results in up-regulation of JUN, FOS, and AP1 in human H&N cell lines (SSC-016, SSC-040, SSC-056, SSC-114, and SSC-116). Increased migration velocities were demonstrated for JUN, FOS, and AP1 in all cell lines- indicative of tumor aggressiveness. AP1 is a transcription factor protein composed of JUN and FOS. AP1 is responsible for cellular processes including differentiation, proliferation and apoptosis. JUN codes for c-Jun, a proto-oncogene, which has been implicated in aggressive breast and lung cancer cells.

INTRODUCTION

Most head and neck cancers arise in squamous cells lining the moist mucosal surfaces of the oral cavity, pharynx, larynx, paranasal sinuses, and nasal cavity. Oral cancer is very common; in fact approximately 3% of cancers in the United States occur in the oral cavity. Oral cancer is the 6th most common type of cancer in men and 12th most common form of cancer in women. Oral squamous cell carcinoma (OSCC) is the most common form of oral cancer, accounting for more than 90% of oral cancer cases. The number one site of occurrence for OSCC is on the lateral and ventral tongue (50%), followed by the floor of the mouth (35%). Tobacco use is implicated in 80% of oral cancers and is the number one risk factor for OSCC.

Head and Neck tumor cells, including OSCC cells, have been found to grow in disproportionately high levels of Nitric Oxide (NO) compared to non-tumor cells. Tumors that express a greater quantity of NO have been found to be more aggressive than their lower (NO) expressing counterparts. Currently, there is sparse information regarding the mechanism of how tumor cells are able to adapt to high levels of NO.

AP1- activator protein 1 is a transcription factor that regulates gene expression in response to a variety of stimuli. AP1 controls a variety of cellular processes including differentiation, proliferation and apoptosis and as a result, promotes tumor cell metastasis. AP1 is a heterodimer composed of proteins belonging to the c-Fos and c-Jun families. FOS codes for c-Fos, while JUN codes for c-Jun.

HYPOTHESIS/OBJECTIVE

Adaptation to NO by five Human Head & Neck (H&N) cancer cell lines will result in up-regulation of and increased cell migration of JUN, FOS, & AP1; genes related to increased metastatic potential.

METHODS

- ***Cell lines***: Five human squamous carcinoma cell lines, SCC016 (tongue), SCC040 (tongue), SCC056 (tongue), SCC114 (FOM -floor of mouth), and SCC116 (alveolar ridge), were studied. Treated cell lines were slowly exposed to increasing concentrations of the nitric oxide donor DETA NONOate, reaching a maximum of 600 mM.
- ***Gene Chip Analysis:*** RNA was isolated from both parental and HNO in squamous cancerous cells, and subjected to a gene chip array analysis (Illumina). Over 48,000 genes were analyzed, and gene expression of the SCC016, SCC040, SCC056, SCC114, and SCC116 HNO cells was compared to the SCC016, SCC040, SCC056, SCC114, and SCC116 Parent cells.
- ***Scratch Assays:*** Cell migration analysis was conducted by seeding cells in microtiter plates etched with three horizontal lines, approximately 15 mm. Cobalt Chloride ($CoCl_2$) was utilized at different concentrations to mimic the hypoxic environment seen in metastasizing tumors (.1M, .5M, 1.0M). A sterile toothpick was used to remove a monolayer of cells. Remaining cells were washed and incubated to allow for cell migration. Cellular mobility was captured using hourly images at 20x magnification. Movement across the cellular gap created with the toothpick scratch was measured in pixels using GNU Image Manipulation Software (GIMP2). Using a ruler, pixels were converted to distance in mm. Migration rates were determined by calculating the distance in mm per hour of cellular movement.

RESULTS

JUN, FOS & AP1 were consistently up-regulated in all five human HNO Adapted squamous cell carcinoma lines (SCC016, SCC040, SCC056, SCC114, and SCC116).

JUN, FOS & AP1 demonstrated faster cell migration velocities in all five human HNO Adapted squamous cell carcinoma lines (SCC016, SCC040, SCC056, SCC114, and SCC116) compared to Parent cell lines.

CONCLUSION

- Nitric oxide (NO) induces change in tumor cells by up-regulating key genes including JUN, FOS, & AP1.
- JUN, FOS, & AP1 were consistently up-regulated in all five human HNO Adapted squamous cell carcinoma lines (SCC016, SCC040, SCC056, SCC114, and SCC116).
- Increased migration velocities were demonstrated compared to the parent cell lines.
- Additional studies are required to determine if any genes that up-regulate and/or down-regulate JUN, FOS & AP1 are common to SCC016, SCC040, SCC056, SCC114, and SCC116 HNO cells compared to the SCC016, SCC040, SCC056, SCC114, and SCC116 Parent cells.

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