# Transmembrane Glycoprotein NMB (GPNMB) Is Overexpressed in Head & Neck (H&N) Squamous Cell Carcinomas (SCC) Exposed To Increased Levels of Nitric Oxide (NO)

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## Introduction

Metastasis is a major cause of death in cancer patients, and a great number of those with head and neck cancer have metastatic disease. Metastasis itself is a complex process that involves a number of highly regulated steps, such as, for example, cell invasion and proliferation.

Furthermore, it has been shown that exposure to nitric oxide (NO) results in stem cell-like properties of cancer cells. These cancer cells that are adapted to high levels of NO (HNO cancer cells) take on a more aggressive phenotype, resulting in adverse consequences in the progression of cancer. Cancer patients that present higher levels of NO are observed to have a poorer chance of survival compared to those with lower levels.

Moreover, HNO cancer cells have higher metastatic potential when compared to their parent cell lines. In this study, it was found that HNO cancer cells overexpress the GPNMB gene, which is believed to have a role in metastasis. GPNMB is a type I transmembrane glycoprotein protein that has been found to be up-regulated in various cancers. A key feature of GPNMB is its tripeptide (Arg-Gly-Asp) RGD motif, which is capable of integrin binding. This activity is important when it comes to the regulation of cell migration, cell adhesion, and other vital processes of metastasis.

## HYPOTHESIS

Adapting SCC cell lines to high levels of NO will result in an increased expression of genes that contribute to the progression of metastasis.

## METHODS

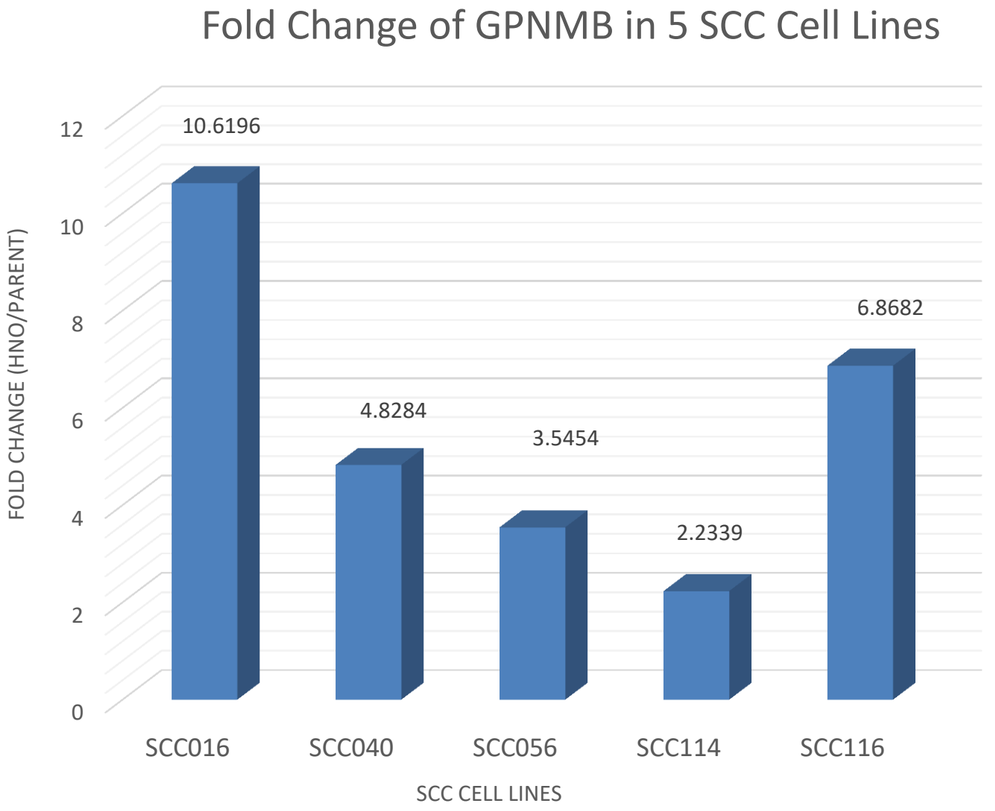
1. In this study, five H&N cell lines were used: SCC016 (tongue), SCC040 (tongue), SCC056 (tongue), SCC114 (floor of mouth), and SCC116 (alveolar ridge). The five SCC cell lines were adapted to high NO levels by exposing the cells to the NO donor DETA-NONOate in increasing amounts until a maximum concentration of 600 mM was reached.
2. RNA was isolated from both HNO and parent cancer cells of the five SCC cell lines. DNA microarrays were then used to compare the gene level expression between the HNO cancer cells and their corresponding parent cells.
3. A proprietary program was developed to compare the obtained gene expression data to a UniProt-GOA association file (Human) in order to find genes belonging to certain Gene Ontology (GO) terms. The GO term used was GO:0005178, which contains genes related to the molecular function of integrin binding.

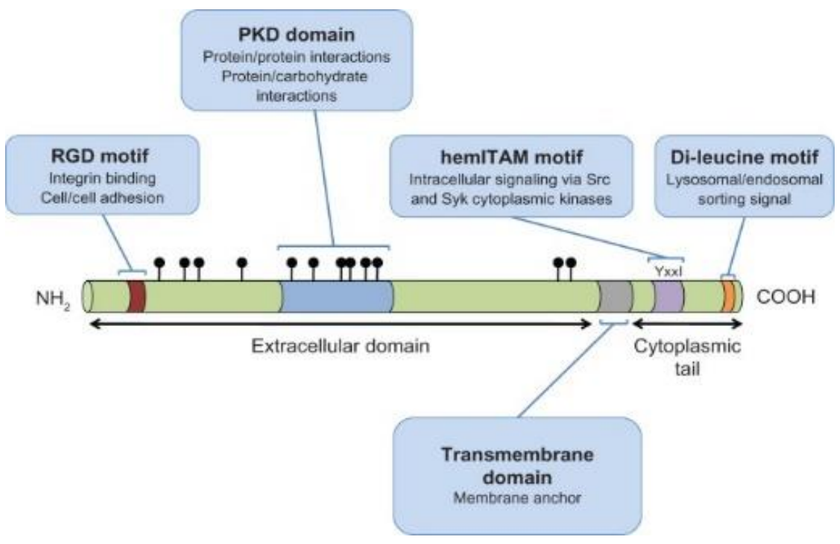
## RESULTS

The genes belonging to the GO term GO:0005178 (molecular function: integrin binding) were obtained with a proprietary program and a UniProt-GOA association file. GPNMB was commonly found in all five SCC cell lines, and it was also seen as being the most up-regulated.

| SCC016 | SCC040 | SCC056 | SCC114 | SCC116 |
|---|---|---|---|---|
| GPNMB | GPNMB | GPNMB | GPNMB | GPNMB |
| ADAMTS5 | CTGF | CIB2 | ITGA5 | LTBP4 |
| EGFL6 | LYN | NOV | ESM1 | EMP2 |
| ECM2 | TGFBI | EMP2 | ITGA2 | LAMA5 |
| EMP2 | CYR61 | ESM1 | CD9 | LYN |
| LYN | LAMA5 | COL5A1 | ADAM10 | ADAM23 |
| CTGF | ANXA7 | ADAM15 | ADAM23 | PTPN2 |
| ITGB5 | ITGA2 | SRC | FBLN1 | ADAM10 |
| EGFR | COL5A1 | ITGB5 | PTPN2 | ADAM17 |
| LAMB1 | GSK3B | LAMA5 | SYK | ITGA6 |
| ADAM17 | ITGB5 | FBLN1 | TIMP2 | CALR |
| ITGA6 | SRC | SYK | | ECM2 |
| ACTN1 | ADAM10 | ADAM17 | | |
| MMP14 | ADAM15 | GSK3B | | |
| COL3A1 | ACTN1 | ANXA7 | | |
| | PTPN2 | ERBB2IP | | |
| | MMP14 | CALR | | |
| | | ITGB6 | | |
| | | ICAM1 | | |
| | | PTPN2 | | |
| | | FN1 | | |
| | | THBS1 | | |
| | | ITGB1 | | |
| | | ITGA6 | | |
| | | ITGA2 | | |
| | | ADAM10 | | |
| | | LAMB1 | | |

GPNMB was observed to be consistently overexpressed in the HNO adapted cells of all five SCC cell lines (SCC016, SCC040, SCC056, SCC114, SCC116).




GPNMB contains an RGD motif in its extracellular domain region, which is recognized by many members of the integrin family. Binding of this ligand motif to integrins can lead to important cell adhesion interactions and other metastatic processes.

## CONCLUSION

Exposure to high levels of NO is correlated with an increase in the expression levels of genes that enhance metastatic potential. In this case, HNO cancer cells were found to overexpress the GPNMB gene. Further investigation is needed to understand the mechanism by which GPNMB contributes to the progression of metastasis. Additional studies with varying concentrations of NO and GPNMB expression may also be useful in determining the gene's significance.

## REFERENCES

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## ACKNOWLEDGMENTS

This study was funded with a VA Merit Review Grant.