

COL11A1 is associated with natural killer cell suppression in HNSCC

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INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) often presents clinically past stage II and has a poor 5- year survival ranging from 52-62%. Late-stage presentation indicates more systemic therapy and radiation in addition to surgery. Previous studies have demonstrated that the numbers of tumor infiltrating lymphocytes (TILs), predict patient prognosis, however, these studies fail to identify the role of natural killer (NK) cells. NK cell number positively correlates with increased survival. HNSCC patients have decreased NK cell infiltration. Despite indications of NK importance in HNSCC, mechanisms of NK suppression are not well understood. We previously reported that collagen type XI alpha I (COL11A1) is overexpressed in HNSCC tumors and contributes to tumor progression. Loss of function mutations in COL11A1 in patients with Marshall or Stickler syndrome are associated with inflammation. Here we test the hypothesis that COL11A1 is associated with NK cell suppression. We determined the putative receptor for COL11A1 and downstream signaling. Further we correlated expression of COL11A1 and its signal transducers with NK cell signatures and activity.

AIMS

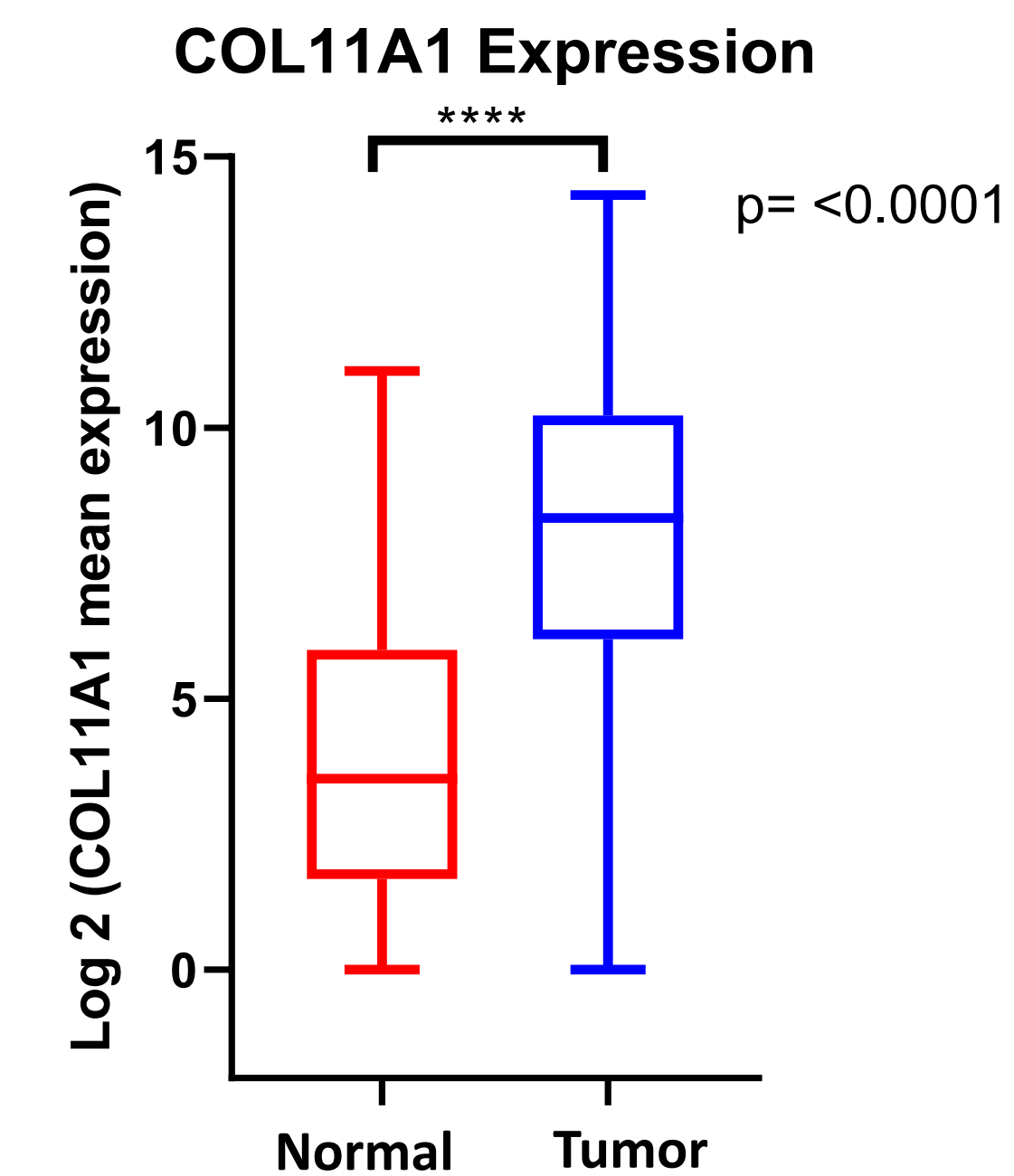
- Investigate relationship between COL11A1 expression and NK cell activity
- Identify potential COL11A1 receptor
- Investigate potential COL11A1 signaling pathways

METHODS

- COL11A1 Gene expression: RNA expression & clinical data from primary tumors (n=522) downloaded from The Cancer Genome Atlas (TCGA). Genes were correlated using Spearman R correlation and statistical significance calculated using Mann-Whitney. Online XenaBrowser tool was used to confirm correlation.
- CIBERSORT was used to predict tumor immune cell infiltrates based on TCGA sequencing data. Following CIBERSORT analysis, NK cell activity was correlated with COL11A1 expression for each patient and plot as NK activity vs. mean COL11A1 expression.
- In vitro* studies were performed using established HNSCC cell line UMSCC-1 and immortalized NK cell line NK92MI. UMSCC-1 cells were transduced with a luciferase reporter and co-cultured with NK92MI cells. NK cytotoxic activity was measured by the amount of luciferase released from UMSCC-1 cells treated with siCOL11A1 or sicontrol. Percent specific lysis was calculated using the equation [(test luc release – spontaneous luc release)/(maximum luc release – spontaneous luc release)] × 100.

RESULTS

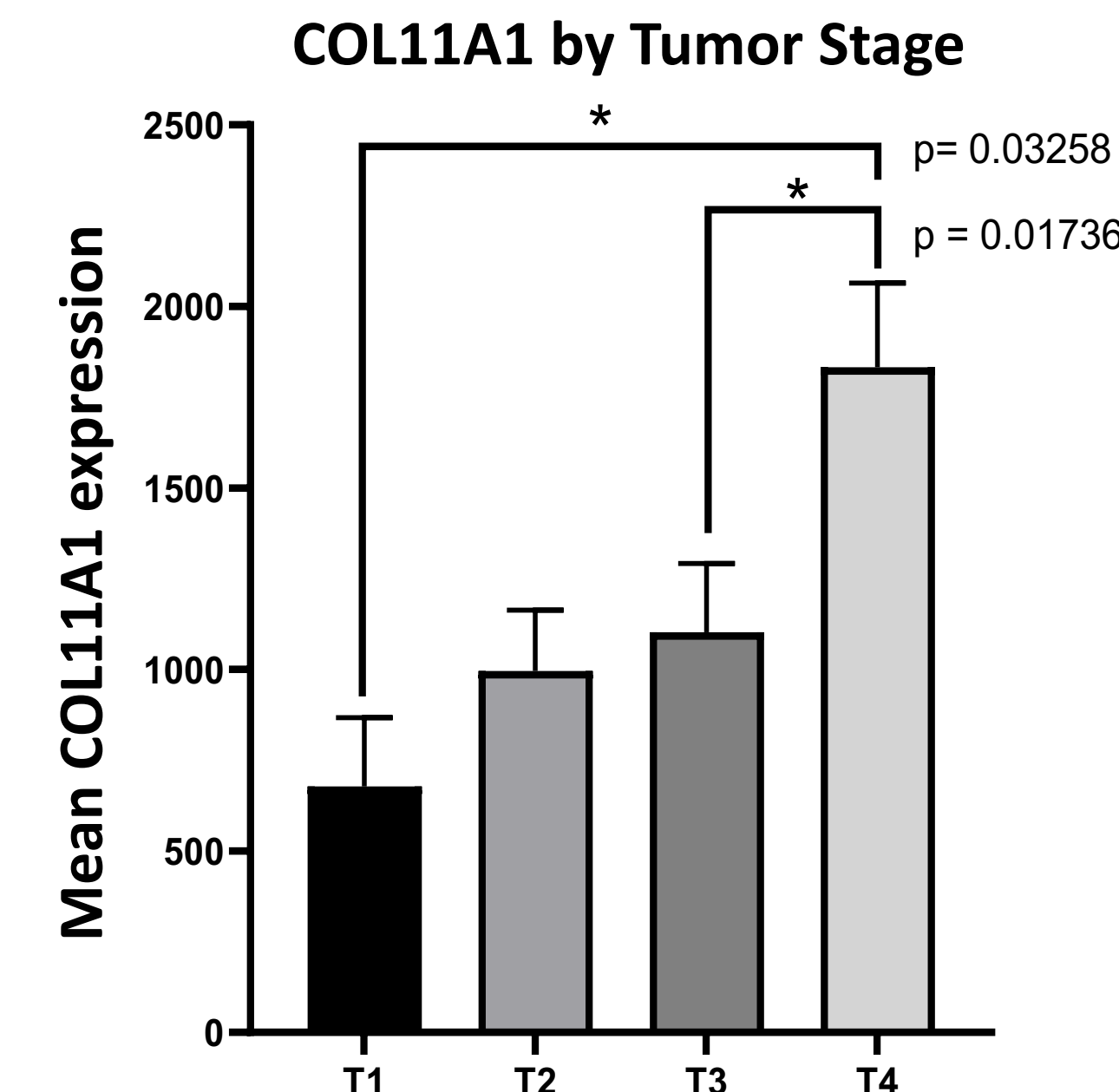
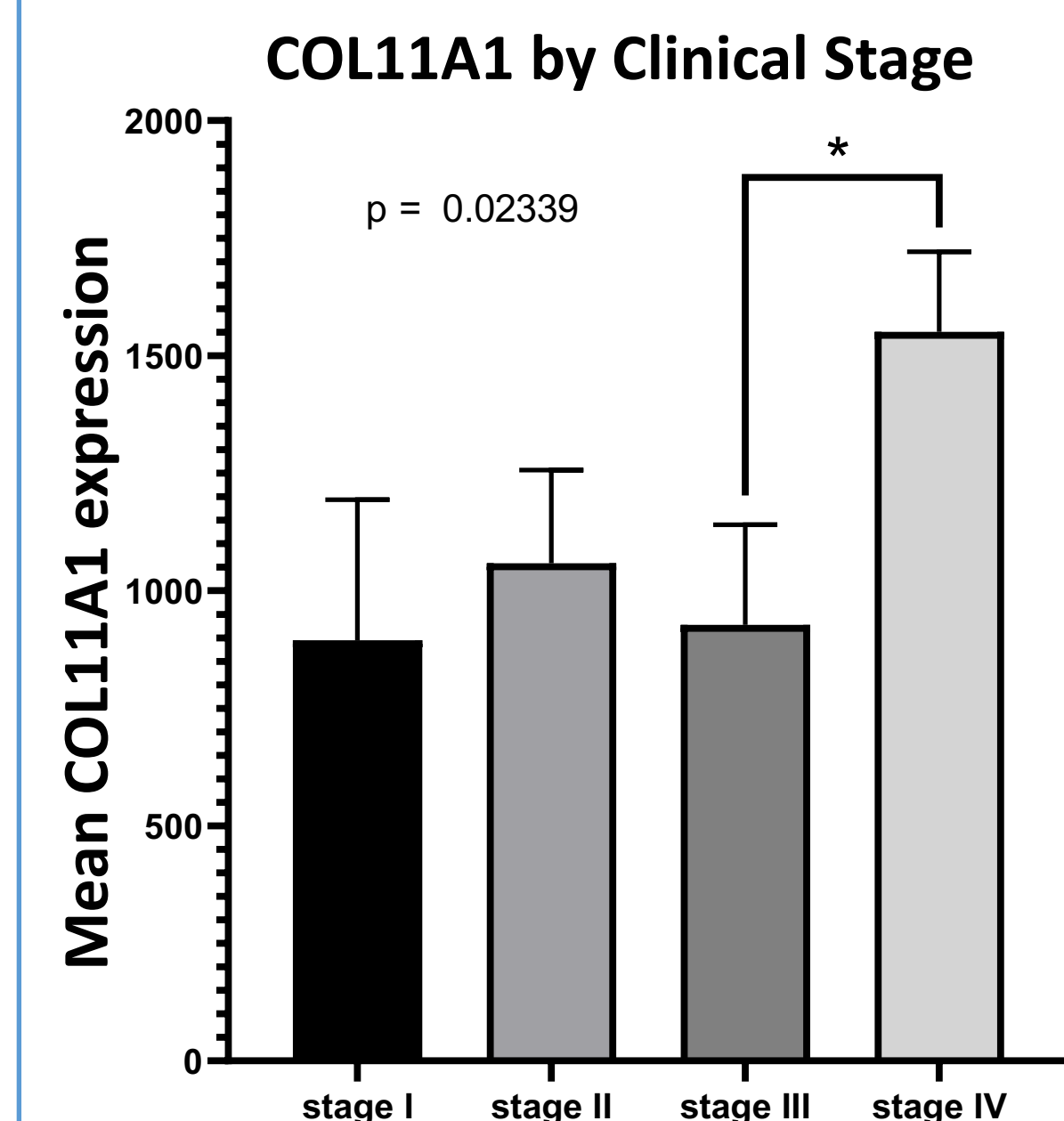
COL11A1 is overexpressed in HNSCC vs. normal adjacent tissue



COL11A1 RNA Expression and clinical data downloaded from TCGA revealed increase in COL11A1 expression compared to normal tissue. Results plotted in GraphPad prism. Mann-Whitney t-test was performed (n=522). Error bars represent ± S.E.M.

COL11A1 is expression increases with tumor progression

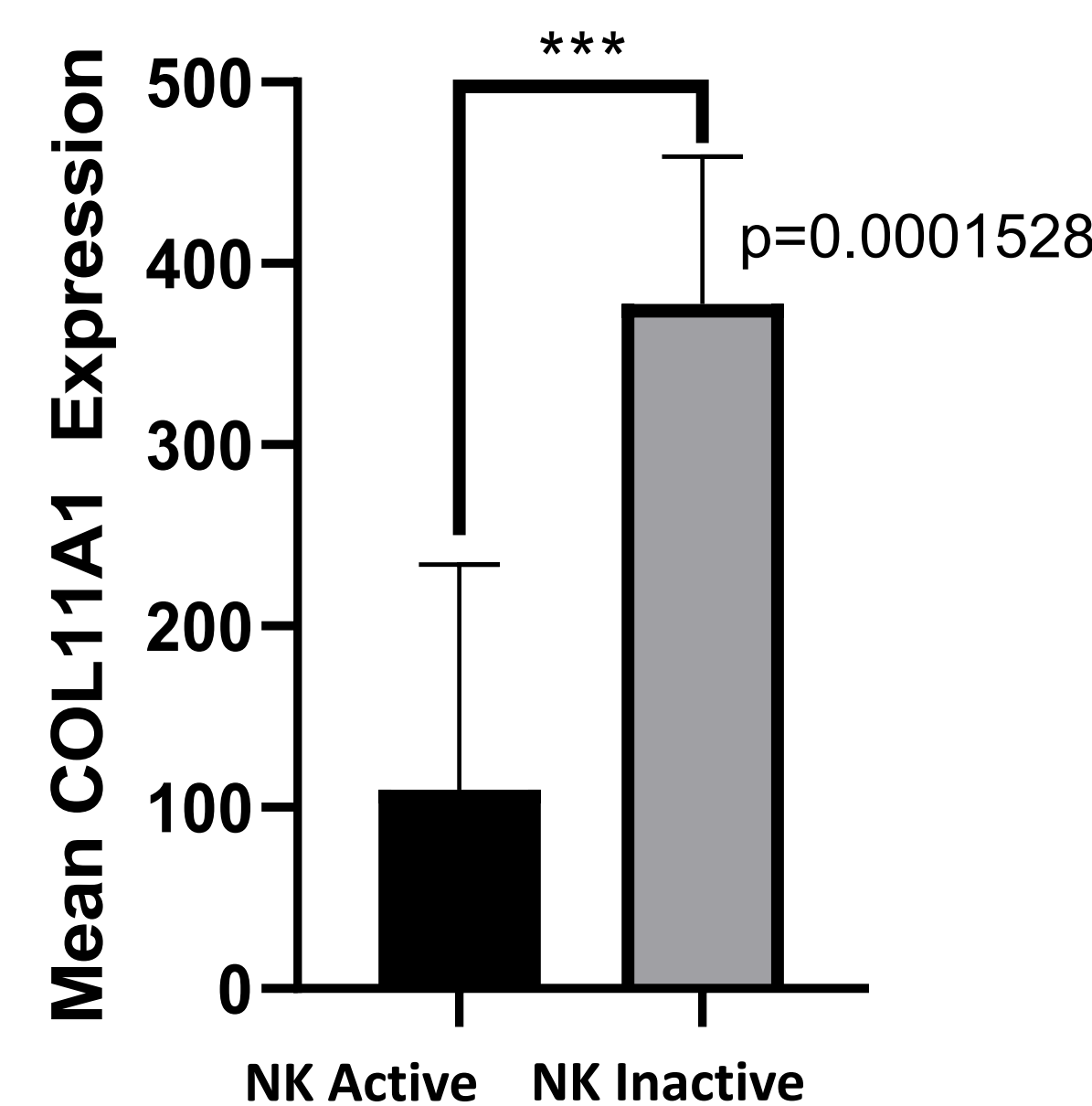
COL11A1 RNA Expression and clinical data downloaded from TCGA revealed increase in COL11A1 expression by tumor and clinical stage. Results plotted in GraphPad prism. Mann-Whitney t-test was performed (n=522). Error bars represent ± S.E.M.



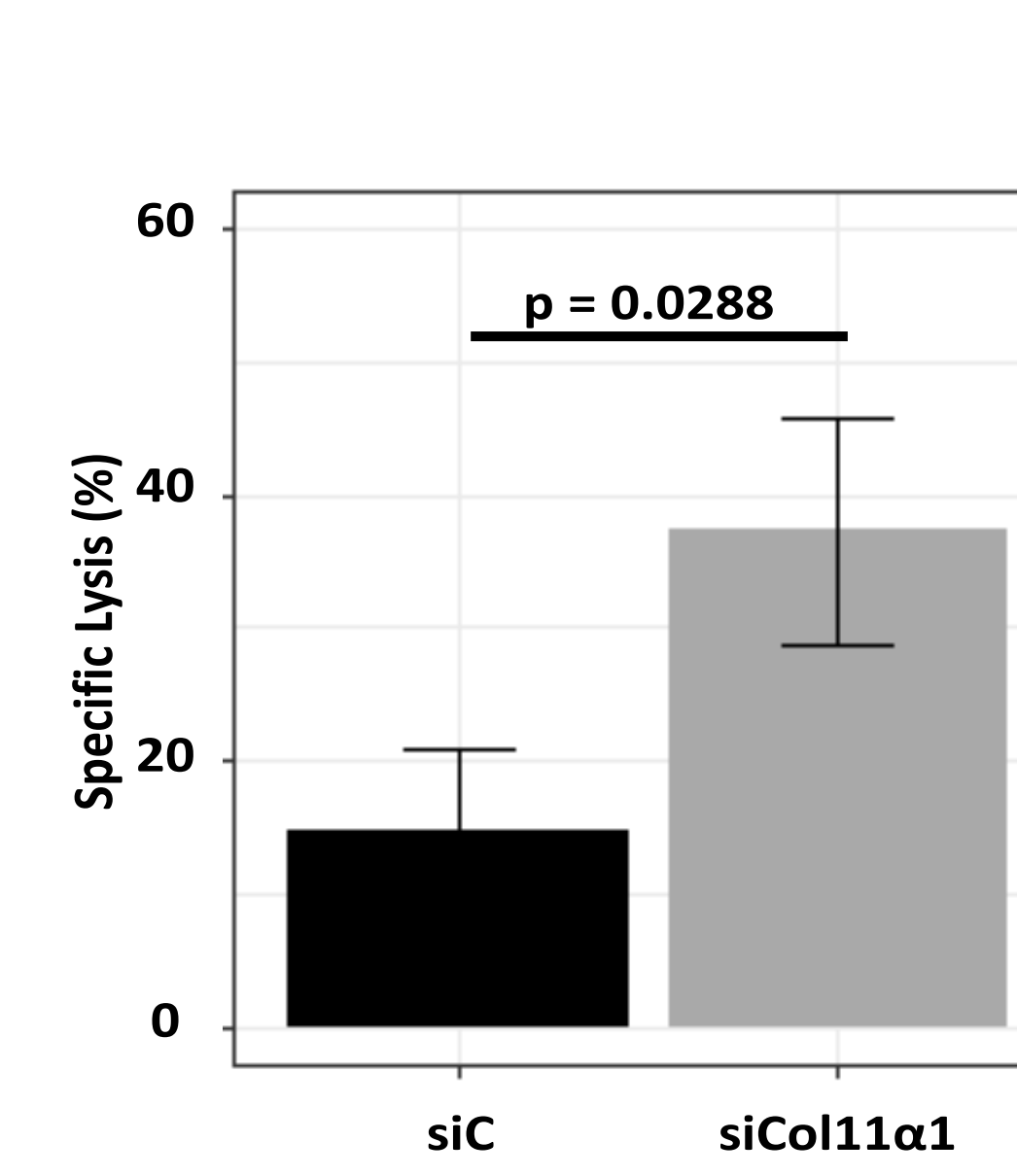
COL11A1 expression suppresses Natural Killer Cell Activity

COL11A1 gene expression levels are increased in patients with lower NK cell activity as demonstrated in (A) according to CIBERSORT analysis and TCGA data. COL11A1 knockdown in luciferase labeled HNSCC cell line increased ability of NK mediated cell lysis *In vitro* (B). COL11A1 knockdown decreased RNA expression of NK evasive markers in HNSCC lines (C). Error bars represent ± S.E.M.

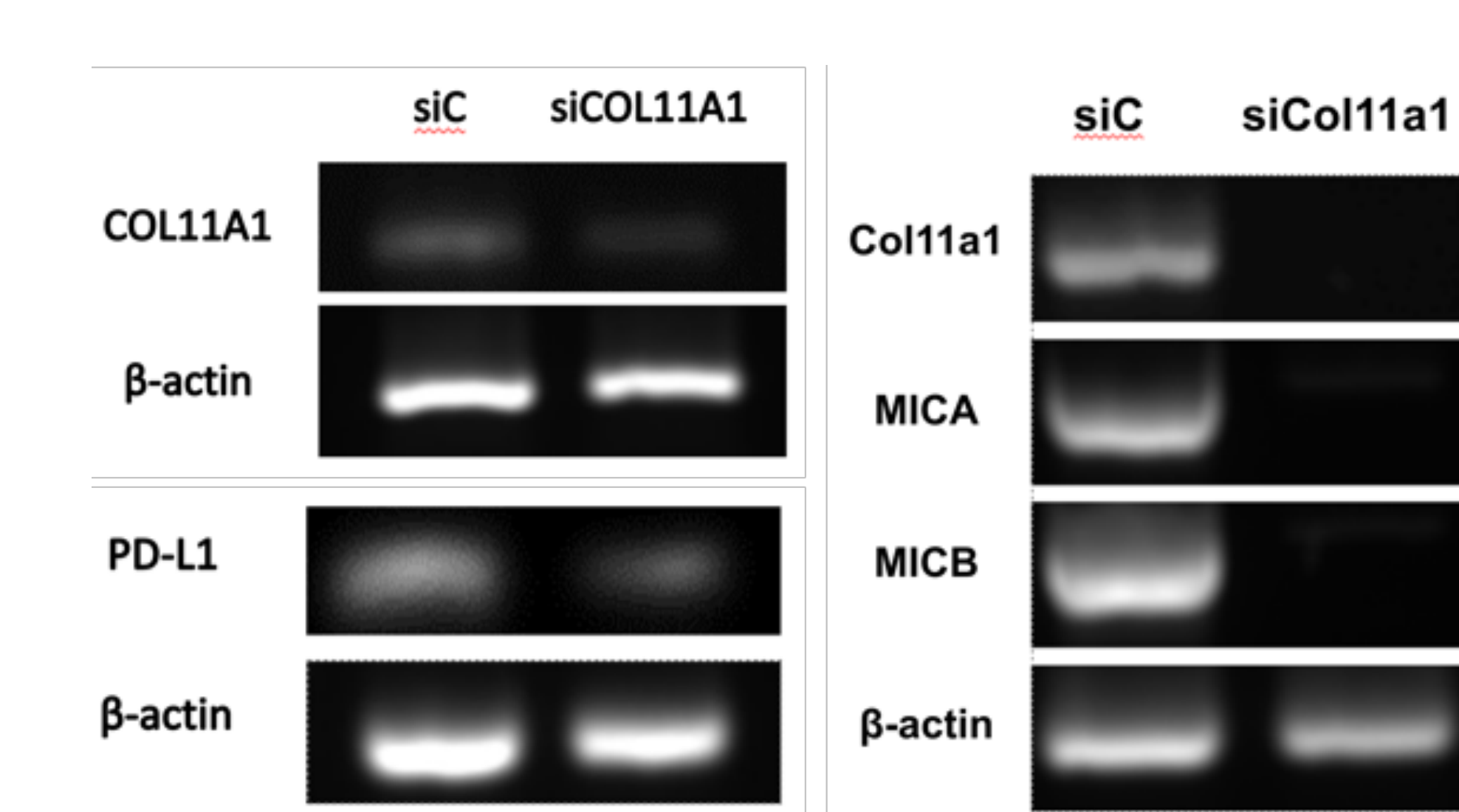
A. Plot of CIBERSORT analysis



B. NK Cytotoxicity of HNSCC line



C. Gel of RT-PCR products in siRNA treated HNSCC cell lines



COL11A1 consensus binding sequence identified in signaling receptor ITGB1

Extracellular matrix signaling were screened for the published COL11A1 consensus binding sequence (GFPER). Integrin Beta 1 (ITGB1), a known receptor upstream of Hippo signaling, was found to have the COL11A1 binding sequence.

ITGB1 GFPGER

MEPWSSHWKTRWLMDFVTTLTALTFLOAREVARGAAPVDVLKALDFHNSPEGISKTTGFCPTRNKRNSKSGDTAYRVSKOALGAPTQKLPF
GOTTFPEDFSILFTVKKPKGIQ6FLLSIYNHGIQI0VEVGRSPVFLFEDHTGKPAPEYPLFRTVNIADGKWHRAISVEKKVTMTMIVDC
KKKTTKPLDRSERAIYDNGITVFGTRILDEVEFEGDIQQFLITGDPKAAAYDYCEHYSPPDCSSAPKAAQEPQIDEYAPEDIDIEYDVEY
GEAEYKEASVTEGPTVTEETIAQTEANIVDDFOBYNYGTMSYQTEAPHVSGTNEPNVVEIITFEYLTGEDYDSQRKNSDITLYENKE
IDGRSDLLVDGDLGEYDFEYKYEYEDKPTSPNNEFGPGVPAETDITETSINGHGAYGEKQKGEPAVYFGLMVEGPPGAGPAGIMGP
PGLQGPPTGPGDGRGPPGRGPGLPAGDGLPGPPGTMMLPFRYGGDGSKGPITSAQEAQAAILQQARIALRGPPGMGLTGRPGVGGP
GSSGAKGESDPPGQPRGVGGPPGPTGKPGKRGRPGADGGRMPGEPGAAGDRGFDGLPGLPDKGHRGERGPGQPGPPGDDGMRGDDG
BIGPRLGLPGEAGPRGLLGRGTGAPGPGMAGVDGPPGKGNMGPQGEPPGPGQGNPGLPGPQGPIGPPEGEKPGQKPLAGLPGA
DGPFGHPGKEGQSGEGALGPPGPGPIGYGPRGVKADGVRLKSGKEGEGDFFGFKGMDLKGDRGEVGGIIGRPGEDGPEGPKGRA
GPTGDGPFGSQAGEKGLGVPLPGYPGRQPKGSTGFPFPFPGANGEGKARGVAGKPGPRGQRTGPRGSRGARGPTGKPGKGTSGGDDG
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ILSSKTRRHTGEMQADADDNILDYSDGMEEIFGSLNSLKQDIEHMKFPMGTQTNPARTCKDLQLSHPDFPDGEYWIIDNPGCSGDSFKVY
CNFTSGGETCIYDPKSEGVRISSWPKEPGSWFSEFKRGLLSYLDVGENSINVMQMTFLKLLTASARQNTYHCHQSAAWYDVSSGSDYD
KALRFLGSDNDEMSYDNNPFITKLYDGCASRKGYEKTVEINTPKIDQVPIVDVMINDFGDNQKQKGFGEVGVPCFLG

CONCLUSIONS

- COL11A1 expression is increased by clinical and tumor stage in HNSCC suggesting its expression influences tumor progression
- COL11A1 may bind ITGB1 and is positively associated with ITB1 downstream pathways and target genes
- Bioinformatic and in vitro results demonstrate decreased NK cell activity in the presence of COL11A1 indicating its role in immune suppression
- Targeting COL11A1 in HNSCC may improve antitumor immune responses in HNSCC by modulating hippo activity and increasing NK cell mediated tumor lysis

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