communications biology

ARTICLE

https://doi.org/10.1038/s42003-023-04437-6 OPEN

# Role of *miR-944/MMP10/AXL*- axis in lymph node metastasis in tongue cancer

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Occult lymph-node metastasis is a crucial predictor of tongue cancer mortality, with an unmet need to understand the underlying mechanism. Our immunohistochemical and real-time PCR analysis of 208 tongue tumors show overexpression of Matrix Metalloproteinase, MMP10, in 86% of node-positive tongue tumors ($n = 79$; $p < 0.00001$). Additionally, global profiling for non-coding RNAs associated with node-positive tumors reveals that of the 11 significantly de-regulated miRNAs, *miR-944* negatively regulates *MMP10* by targeting its 3′-UTR. We demonstrate that proliferation, migration, and invasion of tongue cancer cells are suppressed by *MMP10* knockdown or *miR-944* overexpression. Further, we show that depletion of *MMP10* prevents nodal metastases using an orthotopic tongue cancer mice model. In contrast, overexpression of *MMP10* leads to opposite effects upregulating epithelial-mesenchymal-transition, mediated by a tyrosine kinase gene, *AXL*, to promote nodal and distant metastasis in vivo. Strikingly, *AXL* expression is essential and sufficient to mediate the functional consequence of *MMP10* overexpression. Consistent with our findings, TCGA-HNSC data suggests overexpression of *MMP10* or *AXL* positively correlates with poor survival of the patients. In conclusion, our results establish that the *miR-944/MMP10/AXL*- axis underlies lymph node metastases with potential therapeutic intervention and prediction of nodal metastases in tongue cancer patients.

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*Published online 4 March 2022*

*NAR Cancer, 2022, Vol. 4, No. 1* **1**
*https://doi.org/10.1093/narcan/zcac006*

# *Fusobacterium nucleatum* is associated with inflammation and poor survival in early-stage HPV-negative tongue cancer

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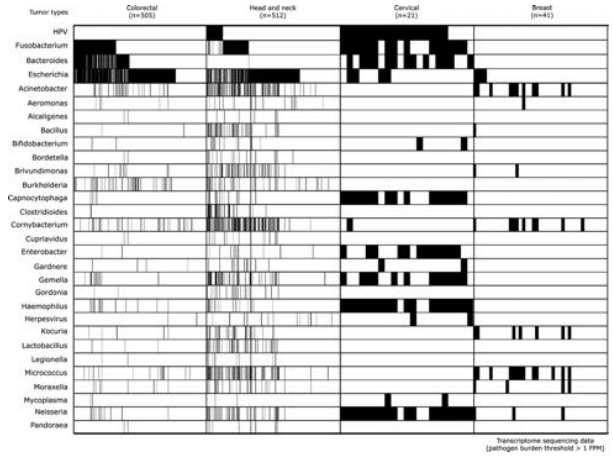
Received November 09, 2021; Revised February 03, 2022; Editorial Decision February 08, 2022; Accepted February 16, 2022

## ABSTRACT

**Persistent pathogen infection is a known cause of malignancy, although with sparse systematic evaluation across tumor types. We present a comprehensive landscape of 1060 infectious pathogens across 239 whole exomes and 1168 transcriptomes of breast, lung, gallbladder, cervical, colorectal, and head and neck tumors. We identify known cancer-associated pathogens consistent with the literature. In addition, we identify a significant prevalence of *Fusobacterium* in head and neck tumors, comparable to colorectal tumors. The *Fusobacterium*-high subgroup of head and neck tumors occurs mutually exclusive to human papillomavirus, and is characterized by overexpression of miRNAs associated with inflammation, elevated innate immune cell fraction and nodal metastases. We validate the association of *Fusobacterium* with the inflammatory markers *IL1B*, *IL6* and *IL8*, miRNAs *hsa-mir-451a*, *hsa-mir-675* and *hsa-mir-486-1*, and *MMP10* in the tongue tumor samples. A higher burden of *Fusobacterium* is also associated with poor survival, nodal metastases and extracapsular spread in tongue tumors defining a distinct subgroup of head and neck cancer.**

## GRAPHICAL ABSTRACT




## INTRODUCTION

The Human Microbiome Project has identified 48 microbial habitats in the human body (1). These microbes maintain balanced symbiotic/commensal relationships or a 'eubiosis' under normal conditions (2). A shift in the eubiotic balance or a 'dysbiosis' can lead to disease. Chronic inflammation, often linked to cancer initiation and pro-

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Yadav *et al. Breast Cancer Research* (2022) 24:97
https://doi.org/10.1186/s13058-022-01597-x

Breast Cancer Research

RESEARCH

Open Access

# Progesterone modulates the *DSCAM-AS1/miR-130a/ESR1* axis to suppress cell invasion and migration in breast cancer

Neelima Yadav[1,2], Roma Sunder[1], Sanket Desai[1,2], Bhasker Dharavath[1,2], Pratik Chandrani[1,2,3], Mukul Godbole[4] and Amit Dutt[1,2*]

## Abstract

**Background:** A preoperative-progesterone intervention increases disease-free survival in patients with breast cancer, with an unknown underlying mechanism. We elucidated the role of non-coding RNAs in response to progesterone in human breast cancer.

**Methods:** Whole transcriptome sequencing dataset of 30 breast primary tumors (10 tumors exposed to hydroxyprogesterone and 20 tumors as control) were re-analyzed to identify differentially expressed non-coding RNAs followed by real-time PCR analyses to validate the expression of candidates. Functional analyses were performed by genetic knockdown, biochemical, and cell-based assays.

**Results:** We identified a significant downregulation in the expression of a long non-coding RNA, *Down syndrome cell adhesion molecule antisense DSCAM-AS1*, in response to progesterone treatment in breast cancer. The progesterone-induced expression of *DSCAM-AS1* could be effectively blocked by the knockdown of progesterone receptor (PR) or treatment of cells with mifepristone (PR-antagonist). We further show that knockdown of *DSCAM-AS1* mimics the effect of progesterone in impeding cell migration and invasion in PR-positive breast cancer cells, while its overexpression shows an opposite effect. Additionally, *DSCAM-AS1* sponges the activity of *miR-130a* that regulates the expression of *ESR1* by binding to its 3′-UTR to mediate the effect of progesterone in breast cancer cells. Consistent with our findings, TCGA analysis suggests that high levels of *miR-130a* correlate with a tendency toward better overall survival in patients with breast cancer.

**Conclusion:** This study presents a mechanism involving the *DSCAM-AS1/miR-130a/ESR1* genomic axis through which progesterone impedes breast cancer cell invasion and migration. The findings highlight the utility of progesterone treatment in impeding metastasis and improving survival outcomes in patients with breast cancer.

**Keywords:** Breast cancer, *DSCAM-AS1*, Estrogen receptor, *miR-130a*, Progesterone, Progesterone receptor

## Introduction

Progesterone and estrogen, naturally occurring hormones, are known to modulate the progression and disease outcome of breast cancer [1–3]. Approximately 70% of breast cancer patients—positive for estrogen receptor (ER) and progesterone receptor (PR)—receive hormone therapy, such as blocking ER to inhibit estrogen signaling, as the first-line treatment for patients with luminal breast

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JBC ARTICLE

⌘ Author's Choice

# Up-regulation of the kinase gene *SGK1* by progesterone activates the AP-1–NDRG1 axis in both PR-positive and -negative breast cancer cells

Received for publication, March 13, 2018, and in revised form, October 13, 2018 Published, Papers in Press, October 18, 2018, DOI 10.1074/jbc.RA118.002894

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Edited by Alex Toker

**Preoperative progesterone intervention has been shown to confer a survival benefit to breast cancer patients independently of their progesterone receptor (PR) status. This observation raises the question how progesterone affects the outcome of PR-negative cancer. Here, using microarray and RNA-Seq–based gene expression profiling and ChIP-Seq analyses of breast cancer cells, we observed that the serum- and glucocorticoid-regulated kinase gene (*SGK1*) and the tumor metastasis–suppressor gene N-Myc downstream regulated gene 1 (*NDRG1*) are up-regulated and that the microRNAs *miR-29a* and *miR-101-1* targeting the 3′-UTR of *SGK1* are down-regulated in response to progesterone. We further demonstrate a dual-phase transcriptional and post-transcriptional regulation of *SGK1* in response to progesterone, leading to an up-regulation of *NDRG1* that is mediated by a set of genes regulated by the transcription factor AP-1. We found that *NDRG1*, in turn, inactivates a set of kinases, impeding the invasion and migration of breast cancer cells. In summary, we propose a model for the mode of action of progesterone in breast cancer. This model helps decipher the molecular basis of observations in a randomized clinical trial of the effect of progesterone on breast cancer and has therefore the potential to improve the prognosis of breast cancer patients receiving preoperative progesterone treatment.**

This work was supported by Wellcome Trust/Department of Biotechnology India Alliance Grant IA/I/11/2500278 (to A. D.), intramural grants (IRB project 2712 to A.D.), and Department of Biotechnology Grant BT/MED/30/VNCI-Hr-RCA/2015 (to A. D.). The funders had no role in the study design, data collection and analysis, decision to publish, or preparation of the manuscript. The authors declare that they have no conflicts of interest with the contents of this article.



This article contains Tables S1–S4 and Figs. S1–S6.

*The microarray raw data were deposited in ArrayExpress under accession number E-MTAB-6742.*

[1] Supported by a research fellowship from the Homi Bhabha National Institute (HBNI), ACTREC-TMC.

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The increasing complexity of multicellular organisms correlates with the increasing number of microRNAs rather than the number of coding genes encoded by the genome (1, 2), reflecting a gradual increase in the extent and intricacy of gene regulation (3). Hierarchically, microRNAs function downstream of transcriptional regulation of genes because microRNAs repress post-transcription of mRNAs (4). However, emerging evidence suggests that transcriptional and post-transcriptional regulation is often highly coordinated (5, 6). Hormones, for instance, have been hypothesized to regulate expression of target genes at the transcriptional and post-transcriptional level (7, 8). Estrogen up-regulates the expression of progesterone receptor (PR)[4] by transcriptionally recruiting estrogen receptor (ER) at the promoter and, post-transcriptionally, by silencing expression of microRNAs targeting the 3′-UTR of *PR* in breast cancer cells (9). A similar example for the *ATP1B1* gene has been reported (10). However, systematic approaches to discern dual-regulated molecular targets of hormones in breast cancer remains poorly understood.

Understanding the molecular basis of clinical phenomena in response to therapeutic interventions has been an important point of intersection between medical and biological sciences. Whereas the clinical benefit of preoperative endocrine therapy is well documented in the literature (11, 12), more recently, we described the first randomized trial with preoperative progesterone resulting in greater than 10% absolute improvement in 5-year disease-free survival among node-positive breast cancer patients (13). Of several hypothesis-generating results from this study, the impact of progesterone on PR-negative patients particularly lends itself to a systematic characterization of molecular changes that progesterone may induce in breast cells.

Gene expression studies probing the targets of progesterone have been performed either restrictively in PR-positive breast cancer cell lines or in the presence of other hormones (14–18). Although few studies suggest a beneficial effect of progesterone, progesterone-responsive genes in PR-negative cells have

[4] The abbreviations used are: PR, progesterone receptor; ER, estrogen receptor; EGFR, epidermal growth factor receptor; GR, glucocorticoid receptor; DMEM, Dulbecco's modified Eagle's medium; M+P, mifepristone + progesterone; sh-NT, short hairpin-nontargeting; p-, phosphorylated; miR, microRNA.

IJC
International Journal of Cancer

# ERBB2 and KRAS alterations mediate response to EGFR inhibitors in early stage gallbladder cancer

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Molecular Cancer Biology

**The uncommonness of gallbladder cancer in the developed world has contributed to the generally poor understanding of the disease. Our integrated analysis of whole exome sequencing, copy number alterations, immunohistochemical, and phospho-proteome array profiling indicates *ERBB2* alterations in 40% early-stage rare gallbladder tumors, among an ethnically distinct population not studied before, that occurs through overexpression in 24% ($n$ = 25) and recurrent mutations in 14% tumors ($n$ = 44); along with co-occurring *KRAS* mutation in 7% tumors ($n$ = 44). We demonstrate that ERBB2 heterodimerizes with EGFR to constitutively activate the ErbB signaling pathway in gallbladder cells. Consistent with this, treatment with *ERBB2*-specific, *EGFR*-specific shRNA or with a covalent EGFR family inhibitor Afatinib inhibits tumor-associated characteristics of the gallbladder cancer cells. Furthermore, we observe an *in vivo* reduction in tumor size of gallbladder xenografts in response to Afatinib is paralleled by a reduction in the amounts of phospho-ERK, in tumors harboring *KRAS* (G13D) mutation but not in *KRAS* (G12V) mutation, supporting an essential role of the ErbB pathway. In overall, besides implicating *ERBB2* as an important therapeutic target under neo-adjuvant or adjuvant settings, we present the first evidence that the presence of *KRAS* mutations may preclude gallbladder cancer patients to respond to anti-EGFR treatment, similar to a clinical algorithm commonly practiced to opt for anti-EGFR treatment in colorectal cancer.**

**Key words:** gallbladder cancer, whole exome sequencing, ErbB pathway, KRAS mutation, targeted therapy

**Abbreviations:** BCA: Bicinchoninic acid assay; NCI-MATCH: NCI-Molecular Analysis for Therapy Choice; PET-CT: Positron Emission Tomography-Computed Tomography; RTK: Receptor tyrosine kinases; SGOL: Segment-of-Gain-Or-Loss; SPSS: Statistical Package for Social Sciences; TKI: Tyrosine Kinase Inhibitors; TMH-TTR: Tumor Tissue repository of Tata Memorial Hospital

Additional Supporting Information may be found in the online version of this article.

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**Conflict of interest:** The authors declare no potential conflict of interest exists.

**Grant sponsor:** Wellcome Trust/DBT India Alliance; **Grant numbers:** IA/I/11/2500278

[Correction added on October 3, 2019 after first online publication: copyright statement updated.]

**DOI:** 10.1002/ijc.31916



**History:** Received 23 Sep 2018; Accepted 27 Sep 2018; Online 10 Oct 2018

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ESMO GOOD SCIENCE BETTER MEDICINE BEST PRACTICE

*Annals of Oncology* 28: 597–603, 2017
doi:10.1093/annonc/mdw636
Published online 19 December 2016

ORIGINAL ARTICLE

# Drug-sensitive *FGFR3* mutations in lung adenocarcinoma

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**Background:** Lung cancer is the leading cause of cancer-related deaths across the world. In this study, we present therapeutically relevant genetic alterations in lung adenocarcinoma of Indian origin.

**Materials and methods:** Forty-five primary lung adenocarcinoma tumors were sequenced for 676 amplicons using RainDance cancer panel at an average coverage of 1500 × (reads per million mapped reads). To validate the findings, 49 mutations across 23 genes were genotyped in an additional set of 363 primary lung adenocarcinoma tumors using mass spectrometry. NIH/3T3 cells over expressing mutant and wild-type *FGFR3* constructs were characterized for anchorage independent growth, constitutive activation, tumor formation and sensitivity to FGFR inhibitors using *in vitro* and xenograft mouse models.

**Results:** We present the first spectrum of actionable alterations in lung adenocarcinoma tumors of Indian origin, and shows that mutations of *FGFR3* are present in 20 of 363 (5.5%) patients. These *FGFR3* mutations are constitutively active and oncogenic when ectopically expressed in NIH/3T3 cells and using a xenograft model in NOD/SCID mice. Inhibition of *FGFR3* kinase activity inhibits transformation of NIH/3T3 overexpressing *FGFR3* constructs and growth of tumors driven by *FGFR3* in the xenograft models. The reduction in tumor size in the mouse is paralleled by a reduction in the amounts of phospho-ERK, validating the *in vitro* findings. Interestingly, the *FGFR3* mutations are significantly higher in a proportion of younger patients and show a trend toward better overall survival, compared with patients lacking actionable alterations or those harboring *KRAS* mutations.

**Conclusion:** We present the first actionable mutation spectrum in Indian lung cancer genome. These findings implicate *FGFR3* as a novel therapeutic in lung adenocarcinoma.

**Key words:** lung adenocarcinoma, actionable mutations, fibroblast growth factor receptor 3, oncogene, FGFR inhibitors, mass spectrometry

## Introduction

Lung cancer is the leading cause of cancer-related deaths worldwide, accounting for over a million deaths annually [1]. Molecularly targeted therapies improve outcome for lung adenocarcinoma patients whose tumors harbor mutant *EGFR* or translocated *ALK*, *RET* or *ROS1*, with an encouraging response for those with mutated *BRAF*, *MET*, *NTRK-1 & 2* and *ERBB2* [2–5]. Such oncogenic somatic alterations though vary across populations/ethnic groups, e.g. *EGFR* mutations are present in over 30% of East Asian lung adenocarcinoma patients, however, they are only found in ∼23%–25% of Indian and 10% of Western lung adenocarcinoma patients [6–8]. Similarly, *KRAS* mutations are present at 60% lower frequency in Indian lung adenocarcinoma patients than compared with the Caucasian population [3, 9, 10].

www.oncotarget.com Oncotarget, 2021, Vol. 12, (No. 6), pp: 578-588

Research Paper

# Molecular characterization of lung squamous cell carcinoma tumors reveals therapeutically relevant alterations

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***Keywords***: lung squamous carcinoma; genetic alterations; druggable mutations; whole exome sequencing; mass spectrometry

***Received***: *November 24, 2020* ***Accepted***: *February 15, 2021* ***Published***: *March 16, 2021*



## ABSTRACT

**Introduction: Unlike lung adenocarcinoma patients, there is no FDA-approved targeted-therapy likely to benefit lung squamous cell carcinoma patients.**

**Materials and Methods: We performed survival analyses of lung squamous cell carcinoma patients harboring therapeutically relevant alterations identified by whole exome sequencing and mass spectrometry-based validation across 430 lung squamous tumors.**

**Results: We report a mean of 11.6 mutations/Mb with a characteristic smoking signature along with mutations in *TP53 (65%), CDKN2A (20%), NFE2L2 (20%), FAT1 (15%), KMT2C (15%), LRP1B (15%), FGFR1 (14%), PTEN (10%)* and *PREX2 (5%)* among lung squamous cell carcinoma patients of Indian descent. In addition, therapeutically relevant *EGFR* mutations occur in 5.8% patients, significantly higher than as reported among Caucasians. In overall, our data suggests 13.5% lung squamous patients harboring druggable mutations have lower median overall survival, and 19% patients with a mutation in at least one gene, known to be associated with cancer, result in significantly shorter median overall survival compared to those without mutations.**

**Conclusions: We present the first comprehensive landscape of genetic alterations underlying Indian lung squamous cell carcinoma patients and identify *EGFR, PIK3CA, KRAS* and *FGFR1* as potentially important therapeutic and prognostic target.**

## INTRODUCTION

Lung cancer is the leading cause of cancer-related deaths across the globe with more than 1.7 million deaths annually [1]. In India, lung cancer contributes to 8.1% of all cancer-related deaths [1]. Non-small cell lung cancer (NSCLC), more common type of lung cancer, accounts for 85% of all lung cancers comprise of two major histological subtypes, adenocarcinoma and squamous cell carcinoma [2]. The adenocarcinoma of the lung arises mostly in patients with no previous significant tobacco exposure, while the squamous subtype is found almost exclusively in former or current smokers [3] with relatively higher overall mutational load [4]. Despite distinct histological and biological characteristics, the two NSCLC subtypes are largely treated with the same chemotherapeutic agents

Oral Oncology 73 (2017) 56–64

Contents lists available at ScienceDirect

Oral Oncology

journal homepage: www.elsevier.com/locate/oraloncology

# Genomic characterization of tobacco/nut chewing HPV-negative early stage tongue tumors identify *MMP10* as a candidate to predict metastases

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ARTICLE INFO

*Article history:*
Received 3 July 2017
Received in revised form 27 July 2017
Accepted 6 August 2017
Available online 12 August 2017

*Keywords:*
HPV-negative early stage tongue cancer
Tobacco/nut chewers
Whole exome and transcriptome sequencing
Nodal metastases
Matrix metalloproteinases

ABSTRACT

*Objectives:* Nodal metastases status among early stage tongue squamous cell cancer patients plays a decisive role in the choice of treatment, wherein about 70% patients can be spared from surgery with an accurate prediction of negative pathological lymph node status. This underscores an unmet need for prognostic biomarkers to stratify the patients who are likely to develop metastases.

*Materials and methods:* We performed high throughput sequencing of fifty four samples derived from HPV negative early stage tongue cancer patients habitual of chewing betel nuts, areca nuts, lime or tobacco using whole exome (n = 47) and transcriptome (n = 17) sequencing that were analyzed using in-house computational tools. Additionally, gene expression meta-analyses were carried out for 253 tongue cancer samples. The candidate genes were validated using qPCR and immuno-histochemical analysis in an extended set of 50 early primary tongue cancer samples.

*Results and conclusion:* Somatic analysis revealed a classical tobacco mutational signature C:G > A:T transversion in 53% patients that were mutated in *TP53*, *NOTCH1*, *CDKN2A*, *HRAS*, *USP6*, *PIK3CA*, *CASP8*, *FAT1*, *APC*, and *JAK1*. Similarly, significant gains at genomic locus 11q13.3 (*CCND1*, *FGF19*, *ORAOV1*, *FADD*), 5p15.33 (*SHANK2*, *MMP16*, *TERT*), and 8q24.3 (*BOP1*); and, losses at 5q22.2 (*APC*), 6q25.3 (*GTF2H2*) and 5q13.2 (*SMN1*) were observed in these samples. Furthermore, an integrated gene-expression analysis of 253 tongue tumors suggested an upregulation of metastases-related pathways and over-expression of MMP10 in 48% tumors that may be crucial to predict nodal metastases in early tongue cancer patients. In overall, we present the first descriptive portrait of somatic alterations underlying the genome of tobacco/nut chewing HPV-negative early tongue cancer, and identify MMP10 as a potential prognostic biomarker to stratify those likely to develop metastases.



## Introduction

Tongue cancer is the most predominant form of oral cancer in developed countries with a varying incidence in developing countries [1]. The major etiological factors associated with tongue cancer include tobacco related products, alcohol and human papilloma virus (HPV) infections [2]. These factors lend to variability across populations, particularly in the Indian subcontinent wherein chewing betel-quid comprising betel leaf (Piper betel), areca nut (*Areca catechu*) and slaked lime (predominantly calcium hydroxide) is a part of the tradition [3]. While tobacco usage shows a 5–25-fold increased risk of cancer [4], HPV infection defines clinical and molecularly distinct subgroups of head and neck squamous cell carcinoma (HNSCC) patients [5]. Such as, HPV-negative tumors are driven by amplification at 11q13, *EGFR* and *FGFR* loci; focal deletions at *NSD1*, *FAT1*, *NOTCH1*, *SMAD4* and *CDKN2A* loci; and, point mutations in *TP53*, *CDKN2A*, *FAT1*, *PIK3CA*, *NOTCH1*, *KMT2D*, and *NSD1* [6,7]. On the other hand, HPV-positive tumors harbor *TRAF3*, *ATM* deletion, *E2F1* amplification, *FGFR2/3* and *KRAS* mutations.

Another unique feature of tongue squamous cell carcinoma (TSCC) compared to other subsites of oral cancer is the occurrence

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http://dx.doi.org/10.1016/j.oraloncology.2017.08.003
1368-8375/

Research Paper

# Notch pathway activation is essential for maintenance of stem-like cells in early tongue cancer

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***Keywords:*** *early stage tongue cancer, exome and transcriptome sequencing, IHC based expression analysis, cancer stem cell-like feature, Notch pathway inhibitors*

***Received:*** *January 25, 2016* ***Accepted:*** *June 07, 2016* ***Published:*** *July 06, 2016*

## ABSTRACT

**Background: Notch pathway plays a complex role depending on cellular contexts: promotes stem cell maintenance or induces terminal differentiation in potential cancer-initiating cells; acts as an oncogene in lymphocytes and mammary tissue or plays a growth-suppressive role in leukemia, liver, skin, and head and neck cancer. Here, we present a novel clinical and functional significance of *NOTCH1* alterations in early stage tongue squamous cell carcinoma (TSCC).**

**Patients and Methods: We analyzed the Notch signaling pathway in 68 early stage TSCC primary tumor samples by whole exome and transcriptome sequencing, real-time PCR based copy number, expression, immuno-histochemical, followed by cell based biochemical and functional assays.**

**Results: We show, unlike TCGA HNSCC data set, *NOTCH1* harbors significantly lower frequency of inactivating mutations (4%); is somatically amplified; and, overexpressed in 31% and 37% of early stage TSCC patients, respectively. HNSCC cell lines over expressing *NOTCH1*, when plated in the absence of attachment, are enriched in stem cell markers and form spheroids. Furthermore, we show that inhibition of NOTCH activation by gamma secretase inhibitor or shRNA mediated knockdown of *NOTCH1* inhibits spheroid forming capacity, transformation, survival and migration of the HNSCC cells suggesting an oncogenic role of *NOTCH1* in TSCC. Clinically, Notch pathway activation is higher in tumors of non-smokers compared to smokers (50% Vs 18%, respectively, *P*=0.026) and is also associated with greater nodal positivity compared to its non-activation (93% Vs 64%, respectively, *P*=0.029).**

**Conclusion: We anticipate that these results could form the basis for therapeutic targeting of NOTCH1 in tongue cancer.**

FULL PAPER

BJC

British Journal of Cancer (2015) 112, 1958–1965 | doi: 10.1038/bjc.2015.121

Keywords: HPV detection; human cancer; next-generation sequencing (NGS)

# NGS-based approach to determine the presence of HPV and their sites of integration in human cancer genome

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**Background:** Human papilloma virus (HPV) accounts for the most common cause of all virus-associated human cancers. Here, we describe the first graphic user interface (GUI)-based automated tool 'HPVDetector', for non-computational biologists, exclusively for detection and annotation of the HPV genome based on next-generation sequencing data sets.

**Methods:** We developed a custom-made reference genome that comprises of human chromosomes along with annotated genome of 143 HPV types as pseudochromosomes. The tool runs on a dual mode as defined by the user: a 'quick mode' to identify presence of HPV types and an 'integration mode' to determine genomic location for the site of integration. The input data can be a paired-end whole-exome, whole-genome or whole-transcriptome data set. The HPVDetector is available in public domain for download: http://www.actrec.gov.in/pi-webpages/AmitDutt/HPVdetector/HPVDetector.html.

**Results:** On the basis of our evaluation of 116 whole-exome, 23 whole-transcriptome and 2 whole-genome data, we were able to identify presence of HPV in 20 exomes and 4 transcriptomes of cervical and head and neck cancer tumour samples. Using the inbuilt annotation module of HPVDetector, we found predominant integration of viral gene *E7*, a known oncogene, at known 17q21, 3q27, 7q35, Xq28 and novel sites of integration in the human genome. Furthermore, co-infection with high-risk HPVs such as 16 and 31 were found to be mutually exclusive compared with low-risk HPV71.

**Conclusions:** HPVDetector is a simple yet precise and robust tool for detecting HPV from tumour samples using variety of next-generation sequencing platforms including whole genome, whole exome and transcriptome. Two different modes (quick detection and integration mode) along with a GUI widen the usability of HPVDetector for biologists and clinicians with minimal computational knowledge.

Human papilloma viral (HPV) infections has been associated with various types of cancer. Epidemiological studies indicate that about 90% of cervical cancers, 90–93% of anal canal cancers, 12–63% of oropharyngeal cancers, 36–40% of penile cancers, 40–64% of vaginal cancers and 40–51% of vulvar cancers are attributable to HPV infection (Munoz *et al*, 2003; Shukla, 2009). Currently, HPV detections are primarily carried out using PCR-based MY09/11 and CPI/II systems (Kleter *et al*, 1998). Other techniques used include the hybridisation-based SPF LiPA method, signal-amplification assays (Hybrid Capture 2 and Cervista) and nucleic-acid-based amplification-like microarray, real-time PCR-based methods (COBAS 4800 real-time test) (Kleter *et al*, 1998; Brink *et al*, 2007; Abreu *et al*, 2012). These technologies come with limitations to detect minor, low-abundance HPV genotypes and a complex mixture of co-infections that can be a negative determinant of the clinical outcome (Mendez *et al*, 2005; Trottier *et al*, 2006). Next-generation sequencing (NGS) technologies overcomes such limitations, as evident from the recently described

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Received 31 July 2014; revised 3 March 2015; accepted 7 March 2015; published online 14 May 2015