

## ‘Functional’ role of ‘Anchoring’ Hemidesmosomal Linker proteins

Oral squamous cell carcinoma (OSCC) is one of the leading cancers in India. Currently, there are no specific biomarkers which can be used for prognostication of human oral cancer. Intermediate filament (cytoskeletal proteins) and their associated proteins have been shown to be aberrantly expressed in various cancers including oral carcinomas. In squamous epithelia like oral cavity, anchoring junctions namely hemidesmosomes (HDs) connect basal epidermal cells to the extracellular matrix. HD linker proteins (BPAG1e and Plectin) anchor intermediate filament proteins like Keratins to the cell surface through  $\beta 4$  integrin. Recent reports indicate that these linker proteins play a role in various cellular processes (eg. cell motility) apart from their known anchoring function. However, the available literature suggests that the role of these proteins in cell motility is context dependent. Moreover, the available information regarding molecular mechanisms underlying BPAG1e and Plectin associated phenotype in cancer is inconsistent. Previously, our laboratory has shown aberrant expression of keratin8/18 in human OSCC. Subsequent work from our laboratory revealed that knockdown of K8 in OSCC derived cell line (AW13516) resulted in alterations in  $\alpha 6\beta 4$  integrin mediated signaling, actin reorganization, reduction in cell motility, invasion and tumorigenicity. Based on these findings, it was hypothesized that linker proteins may have a role in cell motility and neoplastic progression of OSCC.

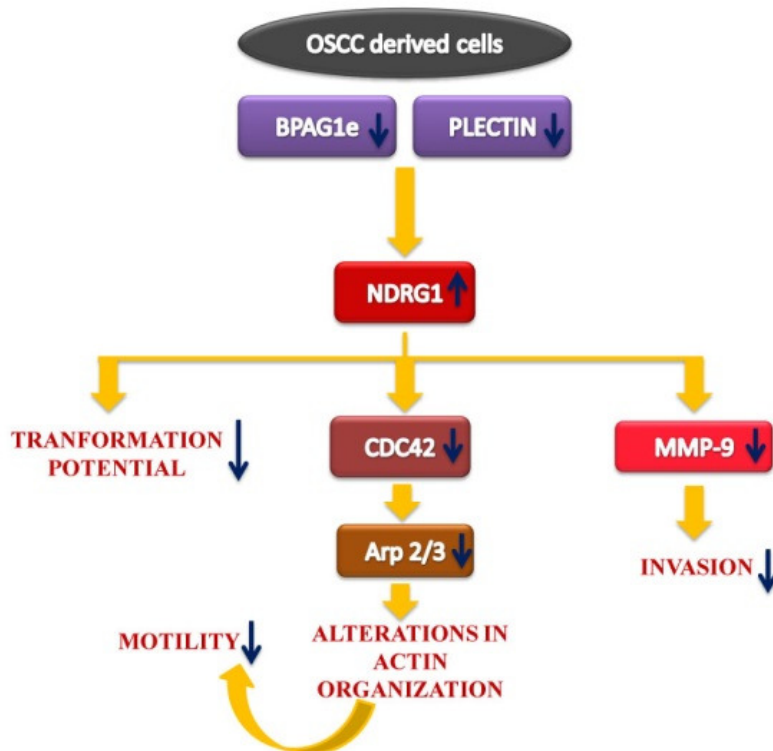


Fig. 1. Schematic representation of the role of Hemidesmosomal linker proteins in oral carcinoma derived cells

To decipher the role of linker proteins in neoplastic progression of OSCC, linker proteins were stably downregulated in OSCC derived AW13516 cells using shRNA technology. Downregulation of HD linker protein(s) in OSCC derived cells resulted in reduced cell migration, accompanied by alterations in actin organization. Further, the results indicated that reduced Cdc42 activity in linker protein(s) knockdown cells led to decreased expression of Arp2/3 proteins, resulting in shorter and fewer filopodia. Furthermore, decreased MMP9 activity led to reduced cell invasion in linker protein(s) knockdown cells. Moreover, loss of these proteins resulted in reduced tumorigenic potential. Next, to decipher the key molecules which may have a role in phenotypic changes observed upon loss of linker proteins in OSCC derived cells, global protein profiling (SWATH) was performed for linker proteins knockdown and respective vector control cells. SWATH analysis demonstrated upregulation of N-Myc downstream regulated gene 1 (NDRG1) in linker proteins downregulated cells as compared to vector control cells. NDRG1 was selected from the list of differentially expressed proteins for further studies, as it plays a role in actin organization, cell migration, cell invasion and tumorigenesis. To understand whether the phenotype observed upon linker proteins knockdown can be reversed by loss of NDRG1, it was stably downregulated in linker proteins knockdown cells. Our experiments revealed that cell motility, cell invasion and tumorigenicity were partially rescued upon NDRG1 loss in linker proteins knockdown background as compared to linker proteins knockdown systems. The similar experiments performed in another tongue SCC derived cell line AW8507 indicated that alterations observed upon loss of BPAG1e and Plectin are not cell line specific.

In conclusion, this study demonstrates that HD linker proteins play a crucial role in regulating cell motility, actin organization, cell invasion and tumorigenicity in OSCC derived cells possibly through NDRG1. Several studies (including present study) by our group have shown functional significance of aberrant expression of Intermediate filament proteins (like Keratins, Vimentin etc.) and their associated proteins (like BPAG1e, Plectin etc.) in oral cancer development. Further studies are necessary on human oral squamous cell carcinomas to investigate whether these proteins can be used as a battery of biomarkers for management of human oral cancer.

*Pratik Rajeev Chaudhari, Sylvania Emlit Charles, Milind Murlidhar Vaidya  
Advanced Centre for Treatment, Research and Education in Cancer (ACTREC),  
Tata Memorial Centre (TMC), Kharghar, Navi Mumbai 410210, India  
and Homi Bhabha National Institute, Training School Complex, Anushakti Nagar, Mumbai 400085, India*

## **Publication**

[Hemidesmosomal linker proteins regulate cell motility, invasion and tumorigenicity in oral squamous cell carcinoma derived cells.](#)

Chaudhari PR, Charles SE, D'Souza ZC, Vaidya MM  
*Exp Cell Res.* 2017 Nov 15