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Desmoglein 3 Acts as a Potential Oncogene in Promoting Cancer Cell Migration and Invasion through Regulating AP-1 and PKC dependent-Ezrin Activation

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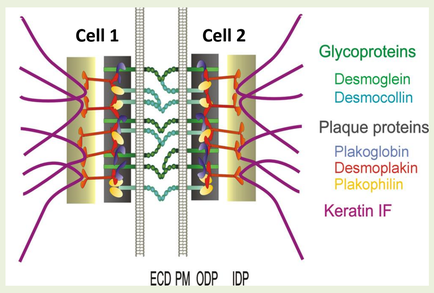
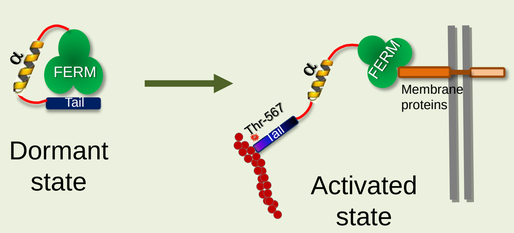
Introduction

Desmoglein 3 (Dsg3) is an adhesion protein in desmosomes that confer strong cell-cell adhesion in epithelia. It is best known as the autoantigen of pemphigus vulgaris, a life threatening blistering disease, caused by autoantibodies targeting Dsg3 which lead to the loss of cell cohesion in the skin and oral mucosa. However, the upregulation of Dsg3 in cancers has been reported recently though the mechanism remains poorly defined. The actin-binding protein Ezrin is an important regulator of membrane cytoskeleton, whereas AP-1 is a dimeric transcription factor composed of proteins including c-Jun/ c-Fos. Both Ezrin and AP-1 are implicated in cancers, especially in the invasive phenotype, and can be activated by Protein kinase C (PKC) and Rho kinase (ROCK). The aim of this study was to investigate the hypothesis that Dsg3 plays a role in regulating Ezrin and AP-1 that could be attributed to cancer cell migration and invasion.

Desmosome structure/ protein components

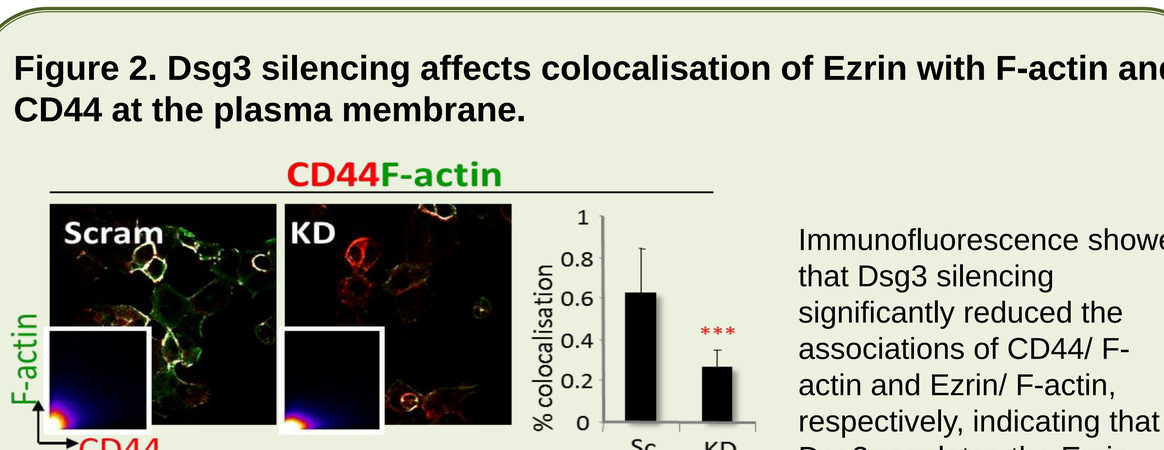


Ezrin activation



Results

Figure 1. Dsg3 colocalises and is physically associated with Ezrin at the plasma membrane.



(A) Confocal images showed partial colocalisation of Dsg3 with Ezrin at the plasma membrane. Arrowheads: Dsg3 expression at the tips of membrane protrusions. (B) Co-IP demonstrated these two proteins formed a complex in Dsg3 dose-dependent manner. Their association was also supported by Proximity Ligation assay and the FRET analysis (data not shown).

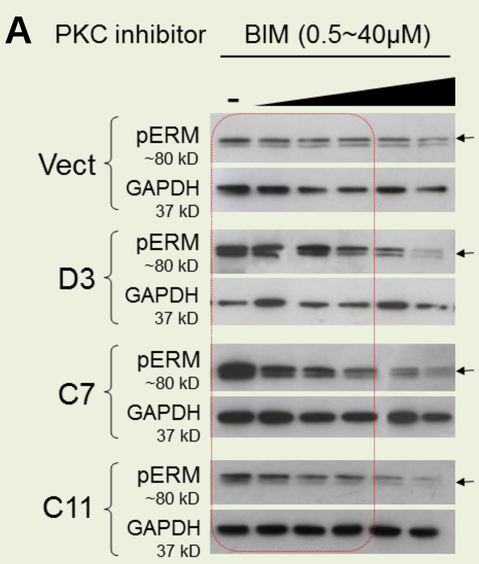
Figure 2. Dsg3 silencing affects colocalisation of Ezrin with F-actin and CD44 at the plasma membrane.

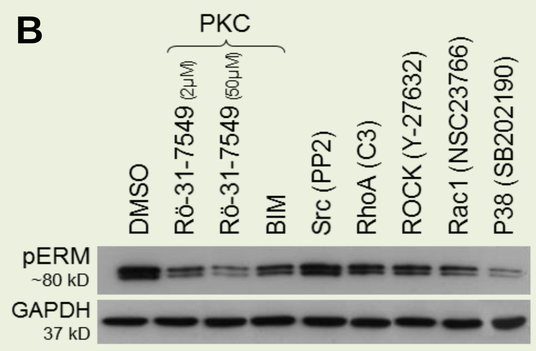
Immunofluorescence showed that Dsg3 silencing significantly reduced the associations of CD44/ F-actin and Ezrin/ F-actin, respectively, indicating that Dsg3 regulates the Ezrin activity at the plasma membrane and its association with other proteins, including the actin cytoskeleton (*p<0.05, ***p<0.001).

Figure 3. Overexpression of Dsg3 enhances Ezrin phosphorylation at T567 (activates Ezrin).

Western blotting analysis indicated that cells with overexpression of Dsg3 showed increased phosphorylation of the ERM (Ezrin-T567/Radixin-T564/Moesin-T558) proteins (*p<0.05, **p<0.01).

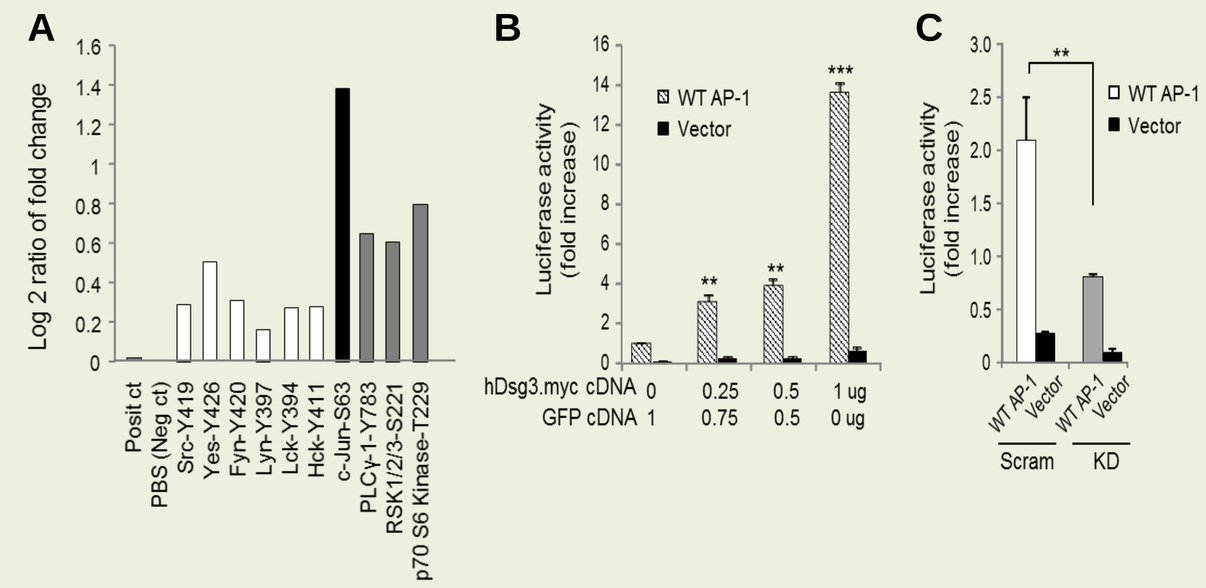
Figure 4. Overexpression of Dsg3 activates Ezrin-T567 that could be abrogated by the PKC and ROCK inhibitions.





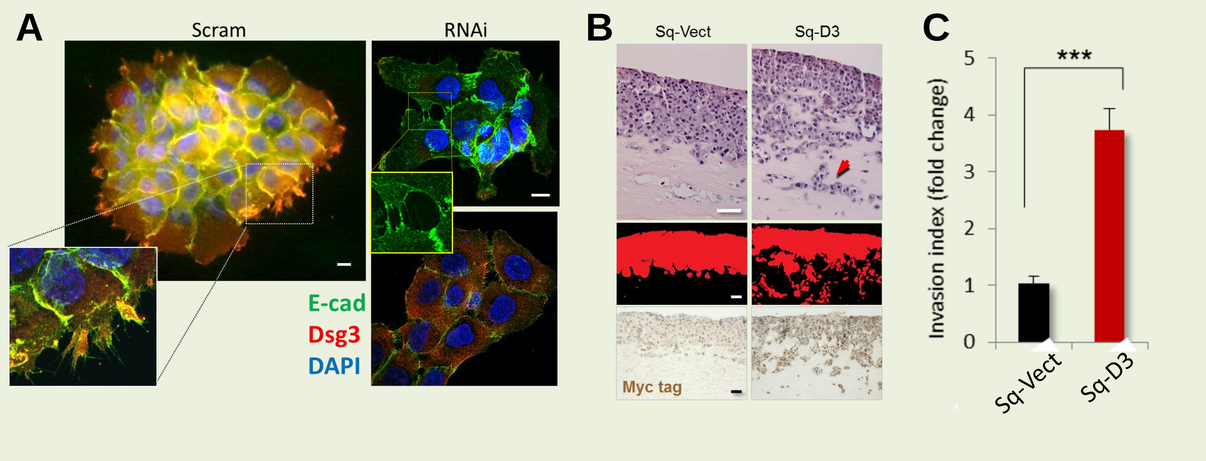
(A) The enhanced Ezrin-T567 in Dsg3 overexpressing cells was inhibited by a PKC inhibitor, BIM, in a dose-dependent manner. (B) The phosphorylation of Ezrin-T567 can also be blocked by various inhibitors including that to PKC, ROCK as well as p38 MAPK.

Figure 5. Overexpression of Dsg3 enhances phosphorylation of c-Jun S63 and activates the AP-1 transcriptional activity.



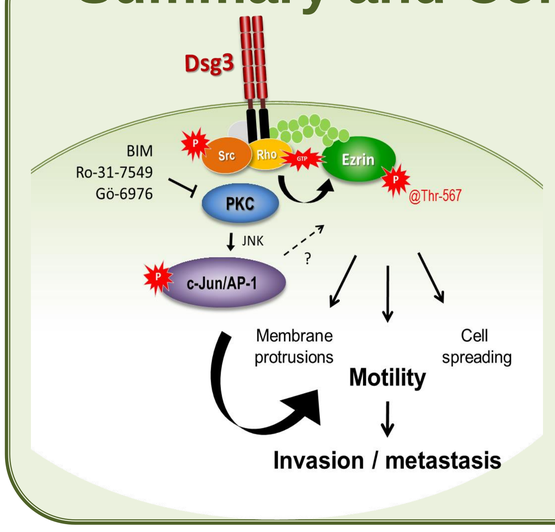
(A) Pospho-kinase array (part of the data) of A431-D3 normalized against to A431-V control cells showed a significant increase of phospho-c-Jun S63 among other kinases. (B) luciferase assay indicated that Dsg3 regulated the AP-1 promoter activity. (C) Dsg3 silencing reduced the luciferase activity more than 2-fold. (**p<0.01, ***p<0.001).

Figure 6. Overexpression of Dsg3 in cancer cell lines induces membrane protrusion and promotes cell migration and invasion.



(A) Fluorescent microscopy of A431-D3 with and without Dsg3 silencing showed that the pronounced membrane protrusions were abolished by Dsg3 depletion with concomitant reduction of E-cadherin. (B) Raft culture indicated that overexpression of Dsg3 in SqCC/Y1 oral cancer line promoted cell invasion. (C) Quantitation of cell invasion in (B) (***p<0.001).

Summary and Conclusion



- Dsg3 associates with Ezrin at the plasma membrane and regulates its activity through T567 phosphorylation.
- The Dsg3 mediated Ezrin activation can be abrogated by PKC inhibition, as well as by several other inhibitors, suggesting it is at least PKC-dependent.
- Our data from this study as well as others suggest that Dsg3 acts as a key regulator for several signal pathways that are essential for the actin-based membrane morphology and cell migration and invasion.

Publications:
1. Brown et al., Oncogene. 2014 May 1;33(18):2363-74. doi: 10.1038/onc.2013.186. Epub 2013 Jun 10.
2. Brown and Wan, Cancers (Basel). 2015 Jan 26;7(1):266-86. doi: 10.3390/cancers7010266. Review.

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