Integrin αvβ3 suppresses expression of the pro-apoptotic mediator PUMA in Breast Cancer cells

Charlie Michael, Isha Bagga, Qi Sun, Jay Desgrosellier

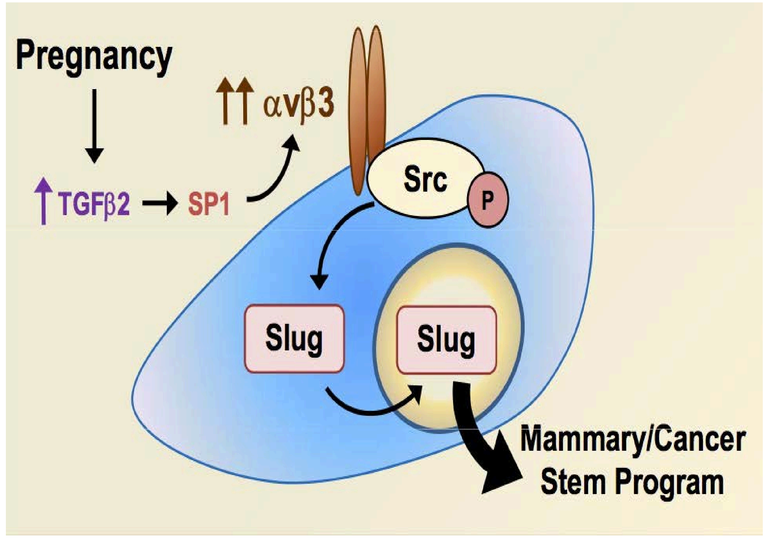
The Department of Pathology, University of California San Diego, 9500 Gilman Drive, La Jolla, California

Abstract

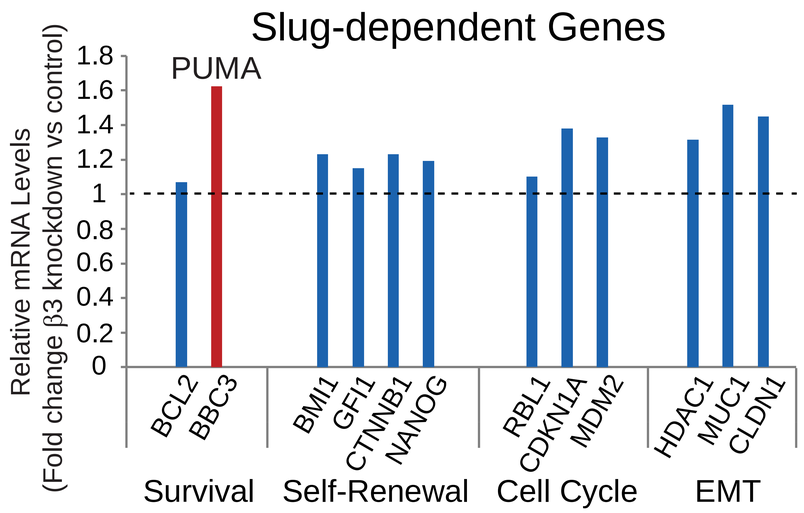
Metastasis is the number one cause of mortality in Breast Cancer patients. Mammary gland stem cells have been linked to more aggressive properties in Breast Cancer, including metastasis. We have shown that the Integrin αvβ3 increases expression of the transcription factor Slug, enhancing stem cell properties in both the mammary gland and aggressive Breast Cancers. This suggests to us that there may be Slug dependent genes that are important for αvβ3's role in Breast Cancer stemness and metastasis.

To identify Slug dependent genes that are regulated by avb3, we screened Breast Cancer cells +/- genetic knockdown of β3 and identified PUMA (p53 upgregulated modulator of apoptosis) as the most upregulated gene in β3 knockdown cells. In a panel of Breast Cancer cell lines expressing ectopic αvβ3 or αvβ3 knockdown, we found that αvβ3 is necessary and sufficient to suppress both PUMA mRNA and protein expression, using qPCR and immunoblot analysis, respectively. This suggests that the αvβ3-Slug pathway may enhance Breast Cancer cell survival by suppressing PUMA, leading to more aggressive metastatic tumors.

Integrin αvβ3's pathway

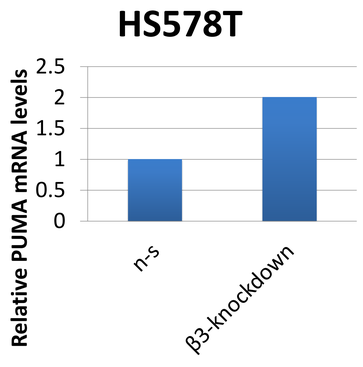


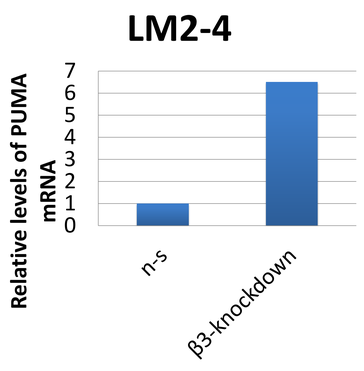
Relative expression of Slug dependent genes

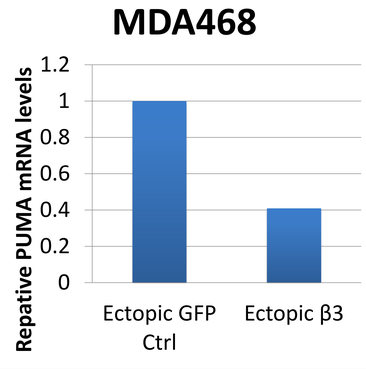


Among all Slug dependent genes, PUMA appears to be the most upregulated when β3 is knocked down

qPCR results

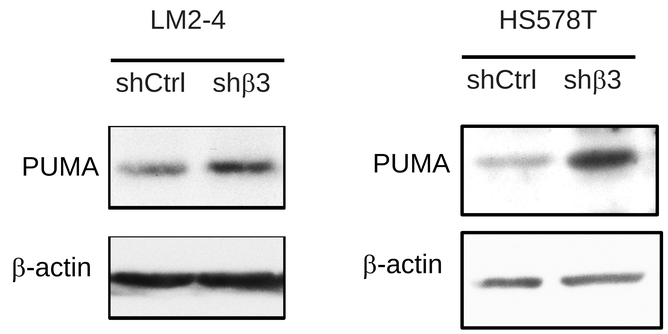






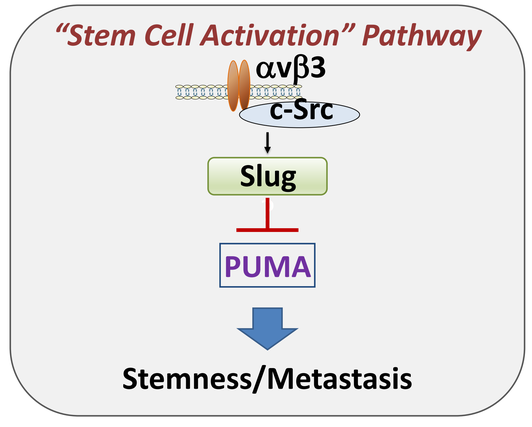
Using qPCR, we analyzed a panel of Breast Cancer cell lines for relative levels of PUMA mRNA. We found that cell lines with β3-knockdown (above) tended to show increased levels of PUMA mRNA, whereas in cell lines without endogenous β3 but with ectopic β3 expression, PUMA mRNA levels were decreased.

Immunoblot Analysis



Immunoblot analysis was used to confirm the differences in PUMA protein levels between control cells and ones with β3 knocked down. This is necessary to confirm that the PUMA mRNA concentrations correlate to the protein concentrations. β-actin was used as a loading control.

PUMA's role in Pathway



Conclusions

Our findings show that Integrin αvβ3 is necessary and sufficient to suppress both PUMA mRNA and protein expression.

Future Research

Further research will continue to investigate a functional role for PUMA in Breast Cancer metastasis and mammary stem cells downstream of αvβ3/Slug signaling.