Fer Tyrosine Kinase Regulates Prostate Cancer Cell Motility Through alpha-Dystroglycan Glycosylation

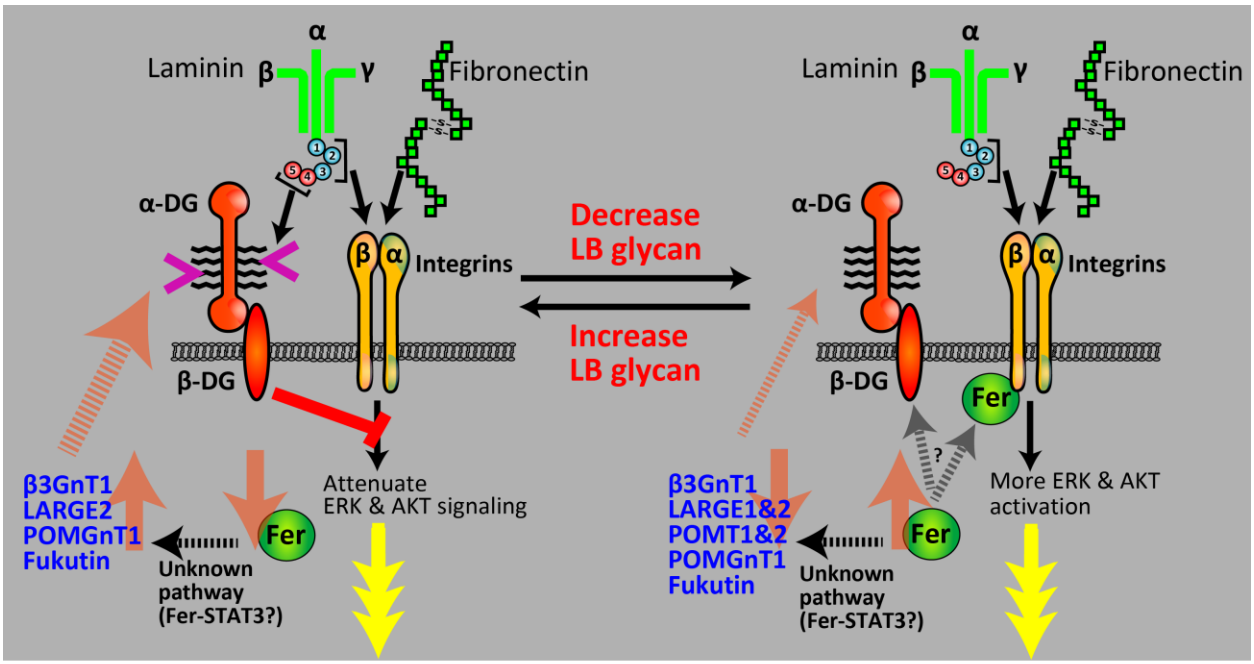
Introduction and Objective: Laminin-binding (LB) glycans of α-dystroglycan (α-DG), which is expressed at the epithelial cell-basement membrane (BM) interface, play an essential role in epithelium development and tissue organization. LB glycans on α-DG expressed on cancer cells suppress tumor progression by attenuating tumor cell migration signal from the BM (1,2). However, mechanisms controlling LB glycan expression are not known yet. Here, we performed siRNA library screening and identified Fer tyrosine kinase, a non-receptor type tyrosine kinase, as a key regulator of LB glycan expression on prostate cancer cells.

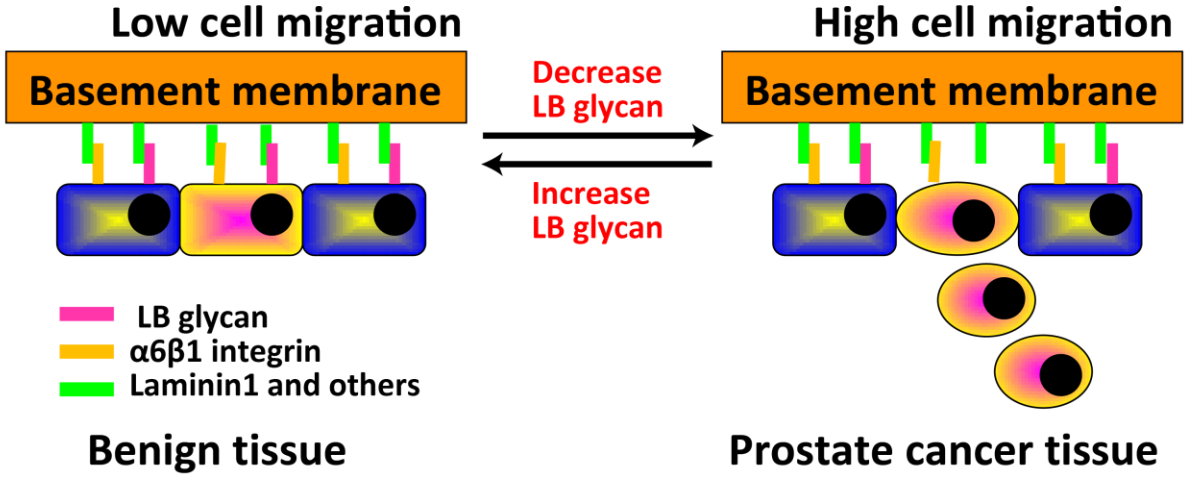
1. Bao X *et al*. *PNAS*; 106:12109-12114 (2009).
2. Shimojo H *et al*. *The Prostate*; 71:1151-1157(2011).

Materials and Methods: Human Kinase siRNAs targeting 704 genes (Ambion) were prepared at the Functional Genomics Facility of Sanford-Burnham Medical Research Institute. Three different siRNAs per gene (a total 2,112 siRNAs) were analyzed in duplicate to avoid effects of non-specific silencing. DU145 prostate cancer cells, which express LB glycans detectable by the IIH6 monoclonal antibody, were used for screening. Next, to investigate function of Fer tyrosine kinase on regulation of LB glycans expression, we performed RT-PCR, FACS, immunoblot analyses, migration and invasion assay by using Fer kinase downregulated or over-expressed DU145 and PC3 prostate cancer cell lines.

Results: Fer overexpression decreased LB glycan expression, while siRNA-mediated knockdown of Fer kinase increased glycan expression on prostate cancer cell lines. Fer expressed more aggressive prostate cancer cell line and human prostate cancer tissues. Loss of Fer kinase function via siRNA increased transcription levels of glycosyltransferases, including POMT1, β3GnT1, and LARGE, which are required to synthesize LB glycans. Consistently, inhibition of Fer expression increases LB glycan thereby decreases cell migration and invasion in the presence of laminin fragment. However, expression or downregulation of Fes, which is highly similar to Fer, does not affect the expression of the LB glycans.

Conclusion: These results indicate that the Fer pathway negatively controls expression of genes required to synthesize LB glycans, thus impairing BM attachment and increasing tumor cell motility. The results also suggest that Fer kinase is an excellent target for downregulation of tumor progression.


Laminin
α
β
γ
Fibronectin
α-DG
β-DG
β
α
Integrins
Decrease
LB glycan
Increase
LB glycan
β3GnT1
LARGE2
POMGnT1
Fukutin
Fer
Unknown
pathway
(Fer-STAT3?)
Attenuate
ERK & AKT signaling
β3GnT1
LARGE1&2
POMT1&2
POMGnT1
Fukutin
?
More ERK & AKT
activation


Low cell migration
High cell migration
Basement membrane
Basement membrane
Decrease
LB glycan
Increase
LB glycan
LB glycan
α6β1 integrin
Laminin1 and others
Benign tissue
Prostate cancer tissue