Supplementary Methods

α5 Integrin Quantitative Polymerase Chain Reaction (QPCR) Assay

Total RNA was isolated from 54 human breast cancer and normal tissues using the RNeasy Mini kit (Qiagen, Valencia CA). For each sample, cDNA was synthesized from 1 µg total RNA using a high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, CA)). The primers for QPCR of human α5 integrin (*ITGA5*) and human glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*; an internal control) were purchased from Integrated DNA Technologies (Coralville, IA) with sequences as follows: *GADPH* forward,, 5'-TTAGGAAAGCCTGCCGGTGACTAA-3'; *GADPH* reverse, 5'-AAAGCATCACCCGGAGGAGAAATC-3'; *ITGA5* forward, 5'-TGCTGGACTGTGGAGAAGACAACA-3'; *ITGA5* reverse, 5'-TCTGGGCAT GGAAAGTGAGGTTCA-3'. The 20 µL PCR reactions included 2 µL of reverse transcription product, 10 µL of iTaq SYBR Green Supermix with ROX (Bio-Rad, Hercules, CA) and 1 µL each of forward and reverse primer (300 nM final concentration). The PCR mixture was incubated at 95° C for 10 minutes, followed by 40 cycles at 95 °C for 15 seconds and at 60 °C for 60 seconds using an ABI7900 Thermal Cycler (Applied Biosystems, Foster City, CA).

Cloning, RNA interference (RNAi) Constructs, and Generation of Stable Cell Lines

We used lentiviral transduction to generate a clone of MDA-MB-231 cells that overexpressed nischarin and a clone of MCF-7 cells in which nischarin expression was silenced by short hairpin RNA (shRNA). Full-length human nischarin was amplified by PCR using the sense primer, 5'-CTAGGAATTCGCCACCATGGAGCAGAAACTGATCTC-3', and the antisense primer, 5'-CTAGGGATCCCTAGCCGGGCCACCTGGCACC-3'. For efficient translation, a Kozak consensus sequence was engineered into the sense primer. The resulting 4545 base

pair PCR product was cloned between the *Eco*RI and *Bam*HI sites of the pCDH-CMV-MCS-EF1-copGFP vector (System Biosciences, Mountain View, CA). The orientation and reading frame of the insert were confirmed by DNA sequencing. Pseudo-viral particles were generated in HEK-293T cells after cotransfecting the pCDH-nischarin plasmid, , which contains a GFP marker, with pCD/NL-BH*ΔΔΔ and pCMV-VSV-G, the latter of which encodes the vesicular stomatis virus glycoprotein under the control of the viral long terminal repeat (9), using the $CaCl_2$ method **[AU: Correct as edited?]**. Second generation lentiviral packaging and envelope particles were kindly provided by Dr. Jakob Reiser (Gene Therapy, Louisiana State University Health Science Center, New Orleans). Supernatants containing lentiviral particles were collected after 48 hours and concentrated by ultracentrifugation at 82,700 × g for 2 hours. Virus particles were reconstituted in 200 μL of serum-free media. Lentiviral titers were measured by fluorescence-activated cell sorting. MDA-MB-231 cells and MDA-MB-231 cells that expressed luciferase plasmids (received from Dr. J.W. Shay, University of Texas Southwestern Medical Center, Dallas) were transduced with GFP- and nischarin-GFP-encoding virus particles to get stable cell clones that expressed GFP and nischarin-GFP. GFP-positive cells were sorted twice in an Aria fluorescence-activated cell sorter at the immunology core facility of the Louisiana State University Health Sciences Center in New Orleans. Western blotting and reverse transcription-PCR were performed to confirm the overexpression of nischarin in MDA-MB-231 cells.

To generate stable clones of MCF-7 cells in which nischarin expression was silenced, we obtained one short hairpin microRNA construct for human nischarin in the lentiviral vector pGIPz, and a control plasmid that expressed turbo GFP-tagged shRNAs from Open Biosystems (Huntsville, AL) (10).

The shRNA sequences were as follows:

Construct 1 (V2LHS_254031):

5’TGCTGTTGACAGTGAGCGCGCCTGTATTCTCTATTCCAATTAGTGAAGCCACAG

ATGTAATTGGAATAGAGAATACAGGCATGCCTACTGCCTCGGA-3’

Control Construct (V2LHS_98703):

5’-TGCTGTTGACAGTGAGCGCCCTGGACCTGTCCTACAACAATAGTGAAGCCACA

GATGTATTGTTGTAGGACAGGTCCAGGTTGCCTACTGCCTCGGA-3’

Virus particles were generated as described above using the same envelope and packaging plasmids in HEK-293T cells. MCF-7 cells (1×10^6) were transduced with virus particles at a multiplicity of 10 viral particles per cell to generate a stable clone of cells in which nischarin expression was silenced. MCF-7 cells transduced with virus particles that expressed GFP were used as a control for our experiments. We performed western blotting and RT-PCR to confirm the silencing of nischarin expression in MCF-7 cells.

Primers

Primers used in the loss of heterozygosity study, for qPCR microdeletion analysis, for nischarin quantitative real-time polymerase chain reaction and for reverse-transcription-polymerase chain reaction of α5 integrin have been listed below.

Primer used for LOH study	Location on chromosome	Primer sequence	Amplicon Size (bp)	Fluorescent Marker
D3S3688	51,838,236-51,838,380	F 5’-CACCACTGCACTCCAG-3’	123-151	6-FAM
		R 5’-TGATTTGTTATTATCTCTTATGGG-3’		
D3S3561	52,321,192-52,321,414	F 5’-TCCTGGGGACTGTGATG-3’	185-229	6-FAM
		R 5’-GGTGACTGGAGGTTCAAG-3’		
D3S3026	51,886,138-51,886,350	F 5’-GCATCTTTGGTCCCAGCTAC-3’	209-226	6-FAM
		R 5’-TAATGGAACACCCTGTGGT-3’		
Primers used for QPCR microdeletion analysis				
QPCR primer 1 (intron 2)	52,489,737-52,489,879	F 5’-TGGACACTTATACGGTGTGTTGGG-3’	143	
		R 5’-TCCTCGCAATCGCAGACCCTTCTA-3’		
QPCR primer 2 (intron 3)	52,491,913-52,492,008	F 5’-TACAGCGACTTCCATGACCTGCAT-3’	96	
		R 5’-ACAATGGAAAGTTGGCCTACGGGT-3’		
QPCR primer 3 (intron 6)	52,506,787-	F 5’TGGCAAGAGCTTTGAAGATGCCC-3’	96	

	52,506,882			
		R 5’-TTGGCATCGCAACCAAAGAGTCC-3’		
Primers used for Nischarin Real-time QPCR (from ABI)				
hNscex1617-ex16	Exon 16	F 5’-CCCCAGGGCTCCTTTGC-3’		
hNscex1617-ex16 M2 (probe)	Exon 16	F 5’-CGAGCGCAGGGCCAG-3’		
hNscex1617-ex16	Exon 17	R 5’-CTCTGCTGGGACCTCCTG-3’		
Primers used in RT-PCR and QPCR assays:				
Human ITGA 5	Exon 28	F 5’-CGGGAGCACCAGCCATTTA-3’		
	Exon 29	R 5’-AGCAGGAGGCCAAACAGGA-3’		
Human Nischarin	Exon 20	F 5’-TGGCATCAGAAAAACACCG-3’	710	
	Exon 22	R 5’ ATGAGGTCATGACCTTGCAC-3’		
Human GADPH (QPCR)		F 5’-TTAGGAAAGCCTGCCGGTGACTAA-3’		
		R 5’-AAAGCATCACCCGGAGGAGAAATC-3’		
Human ITGA5 (QPCR)		F 5’ TGCTGGACTGTGGAGAAGACAACA-3’		
		R 5’-TCTGGGCATGGAAAGTGAGGTTCA-3’		