

Supplementary Material

Table S1. Description of Mouse Skin Carcinogenesis Cell Lines.

Cell Line	Origin	Mutations	Phenotype	Culture Medium	Reference
MCA3D	Primary keratinocytes treated with DMBA	-	E	Hams'F12	[1]
PB	DMBA/TPA induced papilloma	-	E	Hams'F12	[2]
PDV	Primary keratinocytes transformed with DMBA <i>in vitro</i>	H-ras; p53	E	Hams'F12	[3,4]
MSC11 B9	DMBA-induced carcinoma	H-ras; p53	E	DMEM	[5]
MSC11 A5	DMBA-induced carcinoma	H-ras; p53	F	DMEM	[5]
CarcC	DMBA/TPA-induced carcinoma	H-ras	F	DMEM	[6]
CarB	DMBA/TPA-induced carcinoma	H-ras	F	DMEM	[6]

E, epithelial; F, fibroblastic

Table S2. Description of Human SCC Cell Lines

Cell Line	Origin	Culture medium
HaCaT	Normal immortalized keratinocytes	DMEM
HN30	Pharyngeal squamous cell carcinoma	DMEM
HN19	Head and neck squamous cell carcinoma derived from metastatic site: Lymph node	DMEM
HN5	Squamous cell carcinoma of the oral tongue	DMEM
A253	Epidermoid carcinoma from the submaxillary salivary gland	DMEM
Fadu	Pharyngeal squamous cell carcinoma	DMEM
SCC13	Skin squamous cell carcinoma	Keratinocyte-SFM
HEK293T	Human embryonic kidney cells	DMEM

Table S3.- cDNA Constructs for CD44 isoforms

Constructs	Template	Oligonucleotides	Restriction sites	Final Plasmids
CD44sHa	HT1080	5' CCGTTTCGCTCGAATTCATGGACAAGT TT 3' 5'	EcoRI XhoI	pcDNA3 -Ha

		AAGATAATGGTGTAGCTCGAGCACCCC AATCTTCAT 3'		
CD44v3-10-Ha	A253	5' CCGTCGCTCGAATTCCCATGGACAAGT TT3' 5' AAGATAATGGTGTAGCTCGAGCACCCC AATCTTCAT 3'	EcoRI XhoI	pcDNA3 -Ha
CD44v6-10-Ha	HN5	5' CCGTCGCTCGAATTCCCATGGACAAGT TT 3' 5' AAGATAATGGTGTAGCTCGAGCACCCC AATCTTCAT 3'	EcoRI XhoI	pcDNA3 -Ha
CD44v8-10-Ha	HN5	5' CCGTCGCTCGAATTCCCATGGACAAGT TT 3' 5' AAGATAATGGTGTAGCTCGAGCACCCC AATCTTCAT 3'	EcoRI XhoI	pcDNA3 -Ha
CD44sC9-Ha	HN5	5'CCGTCGCTCGAATTCCCATGGACAAG TTT3' 5'ACCTGAATCCTCGAGACTTCTTCGACT GTTGAC3'	EcoRI xhoI	pcDNA3 -Ha
CD44s-eGFP	HT1080	5'CCGTCGCTCGAATTCCCATGGACAAG TTT3' 5'ATAATGGTGTAGGGGTACCACCCCAA TCTTCAT3'	EcoRI KpnI	pEGFP- N1
CD44v3-10-eGFP	A253	5'CCGTCGCTCGAATTCCCATGGACAAG TTT3' 5'ATAATGGTGTAGGGGTACCACCCCAA TCTTCAT3'	EcoRI KpnI	pEGFP- N1
CD44v6-10-eGFP	HN5	5'CTCAAGCTTCGAATTCATGGACAAGTT TTGGTGGCAC3' 5'GATCCCGGGCCCGGGTACCCCCACC CCAATCTTCATGTCCAC3'	EcoRI KpnI	pEGFP- N1
CD44v8-10-eGFP	HN5	5'CCGTCGCTCGAATTCCCATGGACAAG TTT3' 5'ATAATGGTGTAGGGGTACCACCCCAA TCTTCAT3'	EcoRI KpnI	pEGFP- N1
CD44sC9-eGFP	HN5	5'CCGTCGCTCGAATTCCCATGGACAAG TTT3' 5'ACCTGAAGGTACCCAATTCTTCGACT GTTGAC3'	EcoRI KpnI	pEGFP- N1

Table S4.- Wild Type and Mutant Podoplanin cDNA Constructs

Constructs	Template	Oligonucleotides	Restriction sites	Final Plasmids
PDPN-mCherry	PDPN-eGFP	-	EcoRI KpnI	pmCherry-N1
PDPN-Flag	pcDNA3-PDPN [7]	5' AGCTTCGAATTCCTCCGATGTGG 3' 5' TTGCTCACCTCGAGGGCGAC 3'	EcoRI XhoI	pcDNA3-Flag
PDPN-ΔEC-Flag	PDPN-ΔEC-eGFP [8]	5' CGTCAGATCGGTACCCATGTGG 3' 5' TTGAGCGAATTCTCTGAGTCCGGA3' 5' ATGGTTTGGAAATTCGTGACCCTGGTT 3' 5' TTCAGCCTCGAGGGGCGAGTACCT 3'	KpnI EcoRI EcoRI XhoI	pcDNA3-Flag
PDPN-ΔEC QN.N-Flag	pcDNA3-PDPN ΔEC-Flag	QN 5' GTTATGCAAACATGTCGGGAAGGTAC 3' 5' GTTTTGACATGCATAACCACAACGATG 3' N 5' GGAAACTACTCGCCCCTCG 3' 5' GAGTAGTTTCCCGACATGTTTTG 3'	-	pcDNA3-Flag
PDPN-ΔPLAG3-Flag	pcDNA3-PDPNFlag	5' GGTGCCACCAGCGAAGACCGCTATAAG 3' 5' CGCTGGTGGCACCTGGCATGGC 3'	-	pcDNA3-Flag
PDPN-PLAG3m-Flag	pcDNA3-PDPNFlag	5' GTGCCGCAGCTGATGTGGTGGCTCCAGG AACCAGC 3' 5' CTGGAGCCACCACATCAGCTGCGGCAC CTGGCATGG 3'	-	pcDNA3-Flag
PDPN-S/Tm-Flag	pcDNA3-PDPNFlag	5' AGCTTCGAATTCCTCCGATGTGG 3' 5' TTGCTCACCTCGAGGGCGAC 3'	EcoRI XhoI	pcDNA3-Flag
PDPN-PLAG3Tm-Flag	pcDNA3-PDPN-Flag	5' GGTGGCTCCAGGAACCAG 3' 5' GGAGCCACCACATCATC 3'	-	pcDNA3-Flag
PDPN-PLAG4Tm-Flag	pcDNA3-PDPN-Flag	5' GCCAGCTTCAGAAAGCAC 3' 5' GAAGCTGGCAGATCCTC 3'	-	pcDNA3-Flag
PDPN-I1-Flag	pcDNA3-PDPN-Flag	T65-T66 5' CTTGGCAGCTCTGGTGGCAAC 3' 5' CAGAGCTGCCAAGCCAGACTTATAG 3' T70-T76 5' GGCAGCAGCTGTCAACGCTGTAGCAGG	-	pcDNA3-Flag

		CATTCGCA TC3' 5' CCTGCTACAGCGTTGACAGCTGCTGCCA CCAGAGC 3'		
PDPN-I2-Flag	pcDNA3- PDPN-Flag	S98-T100 5' GTCCAGCCGCCGCGCAGCCTCAAACG 3' 5' GCTGCGGCGGCTGGACTTTGTTCTTG 3' S107-T110 5' CACCGCTCACGCCGCGGAGAAAGTGGA TG 3' 5' CTCCGCGGCGTGAGCGGTGGCCACGTTT G 3'	-	pcDNA3- Flag
PDPN- TMCD45-Flag	PDPN- TMCD45-eGFP [9]	5' AGCTTCGAATTCCTCCGATGTGG 3' 5' TTGCTCACCTCGAGGGCGAC 3'	EcoRI XhoI	pcDNA3- Flag
PDPN- TMSYN-Flag	pcDNA3- PDPN-Flag	5'TACTACGTCGACAATGATGATCATCTT GGGAGTGATTTGC3' 5'TACTACTCCGGACATTTTTCGGAAGTA AACTATGATGATGATGAGG3'	-	pcDNA3- Flag
PDPN- TMGPA-Flag	pcDNA3- PDPN-Flag	5' TACTACGTCGACAATAACACTCATTATT TTTGG 3' 5' TACTACTCCGGACATTTTTCGAATACCG TAAGAAATT AAGAGG 3'	-	pcDNA3- Flag
PDPN-G137L- Flag	PDPN-G137L- eGFP [9]	5' AGCTTCGAATTCCTCCGATGTGG 3' 5' TTGCTCACCTCGAGGGCGAC 3'	EcoRI XhoI	pcDNA3- Flag
PDPN-ΔCT- Flag	PDPN- ΔCTeGFP [8]	5' AGCTTCGAATTCCTCCGATGTGG 3' 5' TTGCTCACCTCGAGGGCGAC 3'	EcoRI XhoI	pcDNA3- Flag
PDPN-QN.N- Flag	PDPN-QNN- eGFP [8]	5' AGCTTCGAATTCCTCCGATGTGG 3' 5' TTGCTCACCTCGAGGGCGAC 3'	EcoRI XhoI	pcDNA3- Flag

Table S5.- Oligonucleotides Used for RT-PCR

Name	Oligonucleotides
hs3	5' TTTGCTCCACCTTCTTGACTCC 3'
hs5	5' GATGGAGAAAGCTCTGAGCATC 3'
C5	5' AAGACATCTACCCAGCAAC 3'
v2	5' GATGAGCACTAGTGCTACAG 3'
v3a	5' ACGTCTTCAAATACCATCTC 3'
v3b	5' TGGGAGCCAAATGAAGAAAA 3'
v4	5' TCAACCACACCACGGGCTTT 3'
v5	5' GTAGACAGAAATGGCACCAC 3'
v6	5' CAGGCAACTCCTAGTAGTAC 3'
v7	5' CAGCCTCAGCTCATAACCAGC 3'
v8	5' TCCAGTCATAGTATAACGCT 3'

v9	5' CAGAGCTTCTCTACATCACA 3'
v10	5' GGTGGAAGAAGAGACCCAAA 3'
C9	5' ACCTGAAGGTACCCAATTCTTCGACTGTTGA C 3'
ms5	5' CAACCGTGATGGTACTCGCT 3'
ms3	5' ATGAGTCACAGTGCGGGAAC 3'
Human PDPN Fw	5' CGGGAACGATGTGGAAGGTGTCA 3'
Human PDPN Rv	5' GGGACAGGGCACAGAGTCAGAAAC 3'
Mouse PDPN Fw	5' AAAAACCCTAGCTGCTGAGGCTCCAA 3'
Mouse PDPN Rv	5' ATGGGTCATCTTCCTCCACAGGAAGAGG 3'
β-actin Fw	5' GTGGGCCGCTCTAGGCACCAA 3'
β-actin Rv	5' CTCCTTGATGTCACGCAGGATTTTC 3'
GAPDH Fw	5' TGAAGGTCGGTGTGAACGGATTTGGC 3'
GAPDH Rv	5' CATGTAGGCCATGAGGTCCACCAC 3'

REFERENCES

1. Kulesz-Martin, M.; Kilkenny, A.E.; Holbrook, K.A.; Digernes, V.; Yuspa, S.H. Properties of carcinogen altered mouse epidermal cells resistant to calcium-induced terminal differentiation. *Carcinogenesis* **1983**, *4*, 1367–1377, doi:10.1093/carcin/4.11.1367.
2. Yuspa, S.H.; Morgan, D.; Lichti, U.; Spangler, E.F.; Michael, D.; Kilkenny, A.; Hennings, H. Cultivation and characterization of cells derived from mouse skin papillomas induced by an initiation-promotion protocol. *Carcinogenesis* **1986**, *7*, 949–958, doi:10.1093/carcin/7.6.949.
3. Pons, M.; Cigudosa, J.C.; Rodríguez-Perales, S.; Bella, J.L.; González, C.; Gamallo, C.; Quintanilla, M. Chromosomal instability and phenotypic plasticity during the squamous–spindle carcinoma transition: association of a specific T(14;15) with malignant progression. *Oncogene* **2005**, *24*, 7608–7618, doi:10.1038/sj.onc.1208903.
4. Quintanilla, M.; Haddow, S.; Jones, D.; Jaffe, D.; Bowden, G.T.; Balmain, A. Comparison of ras activation during epidermal carcinogenesis in vitro and in vivo. *Carcinogenesis* **1991**, *12*, 1875–1881, doi:10.1093/carcin/12.10.1875.
5. Burns, P.A.; Kemp, C.J.; Gannon, J.V.; Lane, D.P.; Bremner, R.; Balmain, A. Loss of heterozygosity and mutational alterations of the p53 gene in skin tumours of interspecific hybrid mice. *Oncogene* **1991**, *6*, 2363–2369.
6. Buchmann, A.; Ruggeri, B.; Klein-Szanto, A.J.P.; Balmain, A. Progression of Squamous Carcinoma Cells to Spindle Carcinomas of Mouse Skin Is Associated with an Imbalance of H-ras Alleles on Chromosome 7. *Cancer Res.* **1991**, *51*, 4097–4101.
7. Martín-Villar, E.; Scholl, F.G.; Gamallo, C.; Yurrita, M.M.; Muñoz-Guerra, M.; Cruces, J.; Quintanilla, M. Characterization of human PA2.26 antigen (T1alpha-2, podoplanin), a small membrane mucin induced in oral squamous cell carcinomas. *Int. J. Cancer* **2005**, *113*, 899–910, doi:10.1002/ijc.20656.
8. Martín-Villar, E.; Megías, D.; Castel, S.; Yurrita, M.M.; Vilaró, S.; Quintanilla, M. Podoplanin binds ERM proteins to activate RhoA and promote epithelial-mesenchymal transition. *J. Cell Sci.* **2006**, *119*, 4541–4553, doi:10.1242/jcs.03218.

9. Fernández-Muñoz, B.; Yurrita, M.M.; Martín-Villar, E.; Carrasco-Ramírez, P.; Megías, D.; Renart, J.; Quintanilla, M. The transmembrane domain of podoplanin is required for its association with lipid rafts and the induction of epithelial-mesenchymal transition. *Int. J. Biochem. Cell Biol.* **2011**, *43*, 886–896, doi:10.1016/j.biocel.2011.02.010.