

## REFERENCES

- (1) Tseng, S.C., Concept and application of limbal stem cells. *Eye (Lond)*, **3**(Pt 2) (1989): 141-57.
- (2) Shortt, A.J., et al., Transplantation of ex vivo cultured limbal epithelial stem cells: a review of techniques and clinical results. *Surv Ophthalmol*, **52**(5) (2007): 483-502.
- (3) Balasubramanian, S., et al., Influence of feeder layer on the expression of stem cell markers in cultured limbal corneal epithelial cells. *Indian J Med Res*, **128**(5) (2008): 616-22.
- (4) Joo, C.K. and Y. Seomun, Matrix metalloproteinase (MMP) and TGF beta 1-stimulated cell migration in skin and cornea wound healing. *Cell Adh Migr*, **2**(4) (2008): 252-3.
- (5) Rizvi, A.Z. and M.H. Wong, Epithelial stem cells and their niche: there's no place like home. *Stem Cells*, **23**(2) (2005): 150-65.
- (6) Botchkarev, V.A., et al., Modulation of BMP signaling by noggin is required for induction of the secondary (nontylotrich) hair follicles. *J Invest Dermatol*, **118**(1) (2002): 3-10.
- (7) Grueterich, M., E.M. Espana, and S.C. Tseng, Ex vivo expansion of limbal epithelial stem cells: amniotic membrane serving as a stem cell niche. *Surv Ophthalmol*, **48**(6) (2003): 631-46.
- (8) Koyano, S., et al., Synthesis and release of activin and noggin by cultured human amniotic epithelial cells. *Dev Growth Differ*, **44**(2) (2002): 103-12.
- (9) Mann, I., A Study of Epithelial Regeneration in the Living Eye. *Br J Ophthalmol*, **28**(1) (1944): 26-40.
- (10) Davanger, M. and A. Evensen, Role of the pericorneal papillary structure in renewal of corneal epithelium. *Nature*, **229**(5286) (1971): 560-1.
- (11) Romano, A.C., et al., Different cell sizes in human limbal and central corneal basal epithelia measured by confocal microscopy and flow cytometry. *Invest Ophthalmol Vis Sci*, **44**(12) (2003): 5125-9.

- (12) Schlotzer-Schrehardt, U. and F.E. Kruse, Identification and characterization of limbal stem cells. *Exp Eye Res*, **81**(3) (2005): 247-64.
- (13) Pellegrini, G., et al., Location and clonal analysis of stem cells and their differentiated progeny in the human ocular surface. *J Cell Biol*, **145**(4) (1999): 769-82.
- (14) Chen, Z., et al., Characterization of putative stem cell phenotype in human limbal epithelia. *Stem Cells*, **22**(3) (2004): 355-66.
- (15) Cotsarelis, G., et al., Existence of slow-cycling limbal epithelial basal cells that can be preferentially stimulated to proliferate: implications on epithelial stem cells. *Cell*, **57**(2) (1989): 201-9.
- (16) Lehrer, M.S., T.T. Sun, and R.M. Lavker, Strategies of epithelial repair: modulation of stem cell and transit amplifying cell proliferation. *J Cell Sci*, **111**(Pt 19) (1998): 2867-75.
- (17) Daniels, J.T., et al., Corneal stem cells in review. *Wound Repair Regen*, **9**(6) (2001): 483-94.
- (18) Morrison, S.J., N.M. Shah, and D.J. Anderson, Regulatory mechanisms in stem cell biology. *Cell*, **88**(3) (1997): 287-98.
- (19) Notara, M. and J.T. Daniels, Biological principals and clinical potentials of limbal epithelial stem cells. *Cell Tissue Res*, **331**(1) (2008): 135-43.
- (20) Zhou, S., et al., The ABC transporter Bcrp1/ABCG2 is expressed in a wide variety of stem cells and is a molecular determinant of the side-population phenotype. *Nat Med*, **7**(9) (2001): 1028-34.
- (21) Watanabe, K., et al., Human limbal epithelium contains side population cells expressing the ATP-binding cassette transporter ABCG2. *FEBS Lett*, **565**(1-3) (2004): 6-10.
- (22) de Paiva, C.S., et al., ABCG2 transporter identifies a population of clonogenic human limbal epithelial cells. *Stem Cells*, **23**(1) (2005): 63-73.
- (23) Pellegrini, G., et al., p63 identifies keratinocyte stem cells. *Proc Natl Acad Sci U S A*, **98**(6) (2001): 3156-61
- (24) Yang, A., et al., p63 is essential for regenerative proliferation in limb, craniofacial and epithelial development. *Nature*, **398**(6729) (1999): 714-8.

- (25) Arpitha, P., et al., High expression of p63 combined with a large N/C ratio defines a subset of human limbal epithelial cells: implications on epithelial stem cells. *Invest Ophthalmol Vis Sci*, **46**(10) (2005): 3631-6.
- (26) Di Iorio, E., et al., Isoforms of DeltaNp63 and the migration of ocular limbal cells in human corneal regeneration. *Proc Natl Acad Sci U S A*, **102**(27) (2005): 9523-8.
- (27) Stepp, M.A. and L. Zhu, Upregulation of alpha 9 integrin and tenascin during epithelial regeneration after debridement in the cornea. *J Histochem Cytochem*, **45**(2) (1997): 189-201.
- (28) Pajooohesh-Ganji, A., et al., Integrins in slow-cycling corneal epithelial cells at the limbus in the mouse. *Stem Cells*, **24**(4) (2006): 1075-86.
- (29) Zhang, J., et al., Identification of the haematopoietic stem cell niche and control of the niche size. *Nature*, **425**(6960) (2003): 836-41.
- (30) Hayashi, R., et al., N-Cadherin is expressed by putative stem/progenitor cells and melanocytes in the human limbal epithelial stem cell niche. *Stem Cells*, **25**(2) (2007): 289-96.
- (31) Barbaro, V., et al., C/EBPdelta regulates cell cycle and self-renewal of human limbal stem cells. *J Cell Biol*, **177**(6) (2007): 1037-49.
- (32) Matic, M., et al., Stem cells of the corneal epithelium lack connexins and metabolite transfer capacity. *Differentiation*, **61**(4) (1997): 251-60.
- (33) Watt, F.M. and B.L. Hogan, Out of Eden: stem cells and their niches. *Science*, **287**(5457) (2000): 1427-30.
- (34) Li, W., et al., Niche regulation of corneal epithelial stem cells at the limbus. *Cell Res*, **17**(1) (2007): 26-36.
- (35) Schlotzer-Schreiberdt, U., et al., Characterization of extracellular matrix components in the limbal epithelial stem cell compartment. *Exp Eye Res*, **85**(6) (2007): 845-60.
- (36) Gipson, I.K., The epithelial basement membrane zone of the limbus. *Eye (Lond)*, **3**(Pt 2) (1989): 132-40.

- (37) Shortt, A.J., et al., Characterization of the limbal epithelial stem cell niche: novel imaging techniques permit *in vivo* observation and targeted biopsy of limbal epithelial stem cells. *Stem Cells*, 25(6) (2007): 1402-9.
- (38) Klenkler, B. and H. Sheardown, Growth factors in the anterior segment: role in tissue maintenance, wound healing and ocular pathology. *Exp Eye Res*, 79(5) (2004): 677-88.
- (39) Rizvi, A.Z. and M.H. Wong, Epithelial stem cells and their niche: there's no place like home. *Stem Cells*, 23(2) (2005): 150-65.
- (40) Gandarillas, A. and F.M. Watt, c-Myc promotes differentiation of human epidermal stem cells. *Genes Dev*, 11(21) (1997): 2869-82.
- (41) Koster, M.I., K.A. Huntzinger, and D.R. Roop, Epidermal differentiation: transgenic/knockout mouse models reveal genes involved in stem cell fate decisions and commitment to differentiation. *J Investig Dermatol Symp Proc*, 7(1) (2002): 41-5.
- (42) Frye, M., et al., Evidence that Myc activation depletes the epidermal stem cell compartment by modulating adhesive interactions with the local microenvironment. *Development*, 130(12) (2003): 2793-808.
- (43) Mukhopadhyay, M., et al., Dkk2 plays an essential role in the corneal fate of the ocular surface epithelium. *Development*, 133(11) (2006): 2149-54.
- (44) Lowell, S., et al., Stimulation of human epidermal differentiation by delta-notch signalling at the boundaries of stem-cell clusters. *Curr Biol*, 10(9) (2000): 491-500.
- (45) Nicolas, M., et al., Notch1 functions as a tumor suppressor in mouse skin. *Nat Genet*, 33(3) (2003): 416-21.
- (46) Lowell, S. and F.M. Watt, Delta regulates keratinocyte spreading and motility independently of differentiation. *Mech Dev*, 107(1-2) (2001): 133-40.
- (47) Nguyen, B.C., et al., Cross-regulation between Notch and p63 in keratinocyte commitment to differentiation. *Genes Dev*, 20(8) (2006): 1028-42.
- (48) Okuyama, R., et al., p53 homologue, p51/p63, maintains the immaturity of keratinocyte stem cells by inhibiting Notch1 activity. *Oncogene*, 26(31) (2007): 4478-88.

- (49) Thomas, P.B., et al., Identification of Notch-1 expression in the limbal basal epithelium. *Mol Vis*, 13 (2007): 337-44.
- (50) Nakamura, T., et al., Hes1 regulates corneal development and the function of corneal epithelial stem/progenitor cells. *Stem Cells*, 26(5) (2008): 1265-74.
- (51) Miyazono, K., S. Maeda, and T. Imamura, BMP receptor signaling: transcriptional targets, regulation of signals, and signaling cross-talk. *Cytokine Growth Factor Rev*, 16(3) (2005): 251-63.
- (52) Yamaguchi, K., et al., XIAP, a cellular member of the inhibitor of apoptosis protein family, links the receptors to TAB1-TAK1 in the BMP signaling pathway. *EMBO J*, 18(1) (1999): 179-87.
- (53) Panchision, D.M., et al., Sequential actions of BMP receptors control neural precursor cell production and fate. *Genes Dev*, 15(16) (2001): 2094-110.
- (54) Aberdam, D., et al., Key role of p63 in BMP-4-induced epidermal commitment of embryonic stem cells. *Cell Cycle*, 6(3) (2007): 291-4.
- (55) Zhang, J., et al., Bone morphogenetic protein signaling inhibits hair follicle anagen induction by restricting epithelial stem/progenitor cell activation and expansion. *Stem Cells*, 24(12) (2006): 2826-39.
- (56) Van Mater, D., et al., Transient activation of beta -catenin signaling in cutaneous keratinocytes is sufficient to trigger the active growth phase of the hair cycle in mice. *Genes Dev*, 17(10) (2003): 1219-24.
- (57) He, X.C., et al., BMP signaling inhibits intestinal stem cell self-renewal through suppression of Wnt-beta-catenin signaling. *Nat Genet*, 36(10) (2004): 1117-21.
- (58) Gosselet, F.P., et al., BMP2 and BMP6 control p57 (Kip2) expression and cell growth arrest/terminal differentiation in normal primary human epidermal keratinocytes. *Cell Signal*, 19(4) (2007): 731-9.
- (59) Ichikawa, T., et al., DeltaNp63/BMP-7-dependent expression of matrilin-2 is involved in keratinocyte migration in response to wounding. *Biochem Biophys Res Commun*, 369(4) (2008): 994-1000.

- (60) Boswell, B.A., P.A. Overbeek, and L.S. Musil, Essential role of BMPs in FGF-induced secondary lens fiber differentiation. *Dev Biol*, **324**(2) (2008): 202-12.
- (61) Mohan, R.R., et al., Bone morphogenic proteins 2 and 4 and their receptors in the adult human cornea. *Invest Ophthalmol Vis Sci*, **39**(13) (1998): 2626-36.
- (62) Hayashi, S., et al., Heterozygous deletion at 14q22.1-q22.3 including the BMP4 gene in a patient with psychomotor retardation, congenital corneal opacity and feet polysyndactyly. *Am J Med Genet A*, **146A**(22) (2008): 2905-10.
- (63) Saika, S., et al., Therapeutic effects of adenoviral gene transfer of bone morphogenic protein-7 on a corneal alkali injury model in mice. *Lab Invest*, **85**(4) (2005): 474-86.
- (64) de Caestecker, M., The transforming growth factor-beta superfamily of receptors. *Cytokine Growth Factor Rev*, **15**(1) (2004): 1-11.
- (65) Scherner, O., et al., Endoglin differentially modulates antagonistic transforming growth factor-beta1 and BMP-7 signaling. *J Biol Chem*, **282**(19) (2007): 13934-43.
- (66) Verrecchia, F. and A. Mauviel, Transforming growth factor-beta signaling through the Smad pathway: role in extracellular matrix gene expression and regulation. *J Invest Dermatol*, **118**(2) (2002): 211-5.
- (67) Li, D.Q., S.B. Lee, and S.C. Tseng, Differential expression and regulation of TGF-beta1, TGF-beta2, TGF-beta3, TGF-betaRI, TGF-betaRII and TGF-betaRIII in cultured human corneal, limbal, and conjunctival fibroblasts. *Curr Eye Res*, **19**(2) (1999): 154-61.
- (68) Kinoshita, S., et al., Characteristics of the human ocular surface epithelium. *Prog Retin Eye Res*, **20**(5) (2001): 639-73.
- (69) Joyce, N.C. and J.D. Zieske, Transforming growth factor-beta receptor expression in human cornea. *Invest Ophthalmol Vis Sci*, **38**(10) (1997): 1922-8.

- (70) Hayashida-Hibino, S., et al., The effect of TGF-beta1 on differential gene expression profiles in human corneal epithelium studied by cDNA expression array. *Invest Ophthalmol Vis Sci*, 42(8) (2001): 1691-7.
- (71) Saika, S., TGF-beta signal transduction in corneal wound healing as a therapeutic target. *Cornea*, 23(8 Suppl) (2004): S25-30.
- (72) Zieske, J.D., et al., TGF-beta receptor types I and II are differentially expressed during corneal epithelial wound repair. *Invest Ophthalmol Vis Sci*, 42(7) (2001): 1465-71.
- (73) Chen, Z., et al., Targeted inhibition of p57 and p15 blocks transforming growth factor beta-inhibited proliferation of primary cultured human limbal epithelial cells. *Mol Vis*, 12 (2006): 983-94.
- (74) Schrementi, M.E., et al., Site-specific production of TGF-beta in oral mucosal and cutaneous wounds. *Wound Repair Regen*, 16(1) (2008): 80-6.
- (75) Hay, E.D., An overview of epithelio-mesenchymal transformation. *Acta Anat (Basel)*, 154(1) (1995): 8-20.
- (76) Thiery, J.P. and D. Chopin, Epithelial cell plasticity in development and tumor progression. *Cancer Metastasis Rev*, 18(1) (1999): 31-42.
- (77) Iwano, M., et al., Evidence that fibroblasts derive from epithelium during tissue fibrosis. *J Clin Invest*, 110(3) (2002): 341-50.
- (78) Yang, J. and R.A. Weinberg, Epithelial-mesenchymal transition: at the crossroads of development and tumor metastasis. *Dev Cell*, 14(6) (2008): 818-29.
- (79) Onder, T.T., et al., Loss of E-cadherin promotes metastasis via multiple downstream transcriptional pathways. *Cancer Res*, 68(10) (2008): 3645-54.
- (80) Norton, J.D., ID helix-loop-helix proteins in cell growth, differentiation and tumorigenesis. *J Cell Sci*, 113(Pt 22) (2000): 3897-905.
- (81) Mern, D.S., et al., Targeting Id1 and Id3 by a specific peptide aptamer induces E-box promoter activity, cell cycle arrest, and apoptosis in breast cancer cells. *Breast Cancer Res Treat*.

- (82) Sikder, H.A., et al., Id proteins in cell growth and tumorigenesis. *Cancer Cell*, **3**(6) (2003): 525-30.
- (83) Alani, R.M., et al., Immortalization of primary human keratinocytes by the helix-loop-helix protein, Id-1. *Proc Natl Acad Sci U S A*, **96**(17) (1999): 9637-41.
- (84) Desprez, P.Y., et al., A novel pathway for mammary epithelial cell invasion induced by the helix-loop-helix protein Id-1. *Mol Cell Biol*, **18**(8) (1998): 4577-88.
- (85) Zhang, X., et al., Identification of a novel inhibitor of differentiation-1 (ID-1) binding partner, caveolin-1, and its role in epithelial-mesenchymal transition and resistance to apoptosis in prostate cancer cells. *J Biol Chem*, **282**(46) (2007): 33284-94.
- (86) Asirvatham, A.J., J.P. Carey, and J. Chaudhary, ID1-, ID2-, and ID3-regulated gene expression in E2A positive or negative prostate cancer cells. *Prostate*, **67**(13) (2007): 1411-20.
- (87) Cao, Y., et al., TGF-beta repression of Id2 induces apoptosis in gut epithelial cells. *Oncogene*, **28**(8) (2009): 1089-98.
- (88) Higashikawa, K., et al., DeltaNp63alpha-dependent expression of Id-3 distinctively suppresses the invasiveness of human squamous cell carcinoma. *Int J Cancer*, **124**(12) (2009): 2837-44.
- (89) Yue, J. and K.M. Mulder, Transforming growth factor-beta signal transduction in epithelial cells. *Pharmacol Ther*, **91**(1) (2001): 1-34.
- (90) Moustakas, A., et al., Mechanisms of TGF-beta signaling in regulation of cell growth and differentiation. *Immunol Lett*, **82**(1-2) (2002): 85-91.
- (91) Murray-Zmijewski, F., D.P. Lane, and J.C. Bourdon, p53/p63/p73 isoforms: an orchestra of isoforms to harmonise cell differentiation and response to stress. *Cell Death Differ*, **13**(6) (2006): 962-72.
- (92) Majo, F., et al., Oligopotent stem cells are distributed throughout the mammalian ocular surface. *Nature*, **456**(7219) (2008): 250-4.
- (93) Sun, T.T., S.C. Tseng, and R.M. Lavker, Location of corneal epithelial stem cells. *Nature*, **463**(7284): E10-1; discussion E11.

- (94) Jester, J.V., et al., Transforming growth factor(beta)-mediated corneal myofibroblast differentiation requires actin and fibronectin assembly. *Invest Ophthalmol Vis Sci*, **40**(9) (1999): 1959-67.
- (95) Jester, J.V., W.M. Petroll, and H.D. Cavanagh, Corneal stromal wound healing in refractive surgery: the role of myofibroblasts. *Prog Retin Eye Res*, **18**(3) (1999): 311-56.
- (96) Zhao, X., et al., Adult corneal limbal epithelium: a model for studying neural potential of non-neural stem cells/progenitors. *Dev Biol*, **250**(2) (2002): 317-31.

## APPENDICES

## APPENDIX A

TABLE OF PRIMER SEQUENCES

GENE	ACCESSION	Tm (°C)	SEQUENCE
hABCG2	NM_004827	58	F: AGTTCCATGGCACTGGCCATA R: TCAGGTAGGCAATTGTGAGG
hIntegrin α9	NM_002207	58	F: TGGATCATGCCATCAGTTG R: CCGGTTCTTCTCAGGTTCGAT
hCytokeratin 3	NM_057088	58	F: GGCAGAGATCGAGGGTGTC R: GTCATCCTCGCCTGCTGTAG
hCytokeratin 12	NM_000223	58	F: ACATGAAGAAGAACACGAGGATG R: TCTGCTCAGCGATGGTTCA
hConnexin 43	NM_000165	58	F: CCTTCTTGCTGATCCAGTGGTAC R: ACCAAGGACACCACCAGCAT
hp21cip1	NM_000389	58	F: CCAAGAGGAAGCCCTAATCC R: GAAAAGGAGAACACGGGATG
hp27kip1	NM_004064	58	F: AGTGTCTAACGGGAGGCCCTA R: GTCCATTCCATGAAGTCAGC
hp57kip2	NM_000076	60	F: CACGATGGAGCGTCTGTC R: CTTCTAGGCCTGATCTCT
hBMP4	NM_001202	53	F: GATCCACAGCACTGGCTTGA R: CACTGGTCCCTGGGATGTTG
hNoggin	NM_005450	58	F: CACTACGACCCAGGCTTCAT R: CTCCGCAGCTTCTGCTTAG

hGremlin	NM_013372	58	F: ATCAACCGCTTCTGTTACGG R: ATGCAACGACACTGCTTCAC
hChordin	NM_003741	58	F: CTCTGCTCACTCTGCACCTG R: CCGGTACCATCAAAATAGC
hFollistatin	NM_006350	58	F: TGCCACCTGAGAAAGGCTAC R: ACAGACAGGCTCATCCGACT
hp16lnk4a	NM_000077.3	58	F: AATGGACATTACGGTAGTGGG R: CATCCCCGATTGAAAGAACCC
hld-1	NM_002165.2	57	F: GCTGCTACTCACGCCCTCAAG R: GCCGTTCAGGGTGCTG
hld-2	NM_002166.4	57	F: GAAAAACAGCCTGTCGGACCA R: CCAGGGCGATCTGCAGGT
hE-cadherin	NM_004360.3	58	F: TGCCCAGAAAATGAAAAAGG R: GTGTATGTGGCAATGCGTTC
hN-cadherin	NM_001792.3	58	F: ACAGTGGCCACCTACAAAGG R: CCGAGATGGGTTGATAATG
hFibronectin	NM_212482.1	58	F: CAGTGGGAGACCTCGAGAAG R: TCCCTCGGAACATCAGAAC
hVimentin	NM_003380.2	58	F: GAGAACTTGCCTGTTGAAGC R: GCTTCCTGTAGGTGGCAATC

## APPENDIX B

### WESTERN BLOT REAGENTS

#### 1. 10X Tris Buffered Saline (TBS)

Tris	24.2 g
NaCl	80 g
ddH <sub>2</sub> O	800 mL

Adjust pH to 7.6 with 1 N HCl or 1 N NaOH

Add ddH<sub>2</sub>O to 1 liter

#### 2. 1X Tris Buffered Saline

10X TBS	100 mL
ddH <sub>2</sub> O	900 mL

#### 3. 10X Tris base Glycine

Tris base	30 g
Glycine	144 g
ddH <sub>2</sub> O	800 mL

Adjust pH to 8.3 with 1 N HCl or 1 N NaOH

Add ddH<sub>2</sub>O to 1 liter

#### 4. 1X Running Buffer

10X Tris base Glycine	100 mL
10% SDS	10 mL
dd H <sub>2</sub> O	890 mL

#### 5. Transfer Buffer

10X Tris base Glycine	100 mL
100% Methanol	200 mL
ddH <sub>2</sub> O	700 mL

**6. Washing Buffer (TTBS)**

1X TBS 900 mL

Tween-20 450 µL

**7. Blocking Buffer**

1X TBS 100 mL

Non-fat dry milk 5 g

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