



CONFIRMATION OF DIFFERENTIATION CLUSTERS' AND ENDOGLIN MARKERS PRESET IN PORCINE BUCCAL MUCOSA CELLS

Blanka Borowiec¹, Rut Bryl², Artur Bryja², Paul Mozdziak^{3,4}, Marta Dyszkiewicz-Konwińska^{2,5}

Abstract

Several genes, namely CD44, CD90, CD105 and PCNA may be important in differentiation of porcine mucosa cell cultures. These genes are, inter alia, responsible for cell adhesion to extracellular matrix and its constituent secretion, cytoskeleton organization, epithelial to mesenchymal transition or proper course of DNA replication. A total of 20 pubertal crossbred Landrace gilts bred on commercial farms were used to produce buccal mucosa cultures, which were harvested on the 7th, 15th and 30th day after initiation of the culture. Expression levels of CD44, CD90, CD105 and PCNA were evaluated employing Real-Time Quantitative Polymerase Chain Reaction. CD44, CD90 and PCNA showed an unchanged expression pattern. Expression of CD44 on day 7 was the highest of all factors measured. The greatest difference between the measurement on 7th and 30th day was found in the PCNA gene. These results broaden the understanding of the transcriptome changes in porcine buccal mucosa cells for the duration of in vitro cultivation. Nevertheless, it is very important to consider that the in vitro conditions do not fully reflect the changes taking place in the living organism. It appears that tissues of the oral cavity possess high regenerative potential, and constitute suitable model for wound healing investigation.

Running title: Confirmation of differentiation clusters' and endoglin markers preset in porcine buccal mucosa cells

Keywords: cluster of differentiation, endoglin, cell culture, sus scrofa

¹Department of Histology and Embryology, Poznań University of Medical Sciences, Poznań, Poland

²Department of Anatomy, Poznań University of Medical Sciences, Poznań, Poland

³Physiology Graduate Program, North Carolina State University, Raleigh, USA

⁴Prestage Department of Poultry Science, North Carolina State University, Raleigh, USA

⁵Department of Biomaterials and Experimental Dentistry, Poznań University of Medical Sciences, Poznań, Poland

* **Correspondence:** m.dyszkiewicz@ump.edu.pl

Full list of author information is available at the end of article

Introduction

Pigs are viewed as model organisms for the purpose of translational research, as some morphological similarities between pigs and humans have been clearly noted, such as muscle alignment in the fetal phase, the same thoracic and abdominal organs with only small differences [1] and similar epidermis, with a comparable thickness [2]. Furthermore, numerous genetic and physiological characteristics make pig one of the most versatile and useful animal models. 112 porcine proteins containing amino acids related to corresponding diseases in human genome were identified [3], including genes associated with multifactorial diseases such as diabetes (PPP1RA, SLC30A8, ZNF615), obesity (ADRB3, SDC3) and Alzheimer's disease (including TUBD1, BLMH, PLAUI) [4]. Physiologically, CD molecules can act in many different ways, often as ligands or cell receptors involved in signaling. There are exceptions among CDs, that are not involved in signaling, but may have other roles, such as cell adhesion [5,6]. CD44, also known as Pgp-1 or the Hermes antigen, is an integral cell membrane glycoprotein. It has a role in cell adhesion to extracellular matrix, lymphocyte activation and lymph node homing [7]. Cluster of Differentiation 90, also known as CD90 or Thy-1, is a highly N-glycosylated surface protein anchored to glycosphosphatidylinositol (GPI). CD90 can be used as a marker for the axonal processes of mature neurons and a marker for various types of stem cells. CD90 also initiated the creation of the immunoglobulin superfamily [8,9]. Furthermore, endoglin (ENG), also known as CD105, is a homodimeric membrane glycoprotein found in the vascular endothelium. It can be localized in activated lymphoblasts and monocytes in the presence of leukemia, as well as in bone marrow progenitor cells. Moreover, CD105 binds TGFB1 with high affinity as it is an important component of the transforming growth factor beta (TGFB) receptor complex [10,11]. Finally, proliferating cell nuclear antigen (PCNA) is an important element used in DNA replication, encoding the necessary accessory protein. PCNA was originally identified as a nuclear protein through the use of the immunofluorescence technique, with its appearance found to be dependent on the proliferative state of the cell [12,13]. It is also known that it recruits and retains many enzymes in the replication fork, which are required for DNA repair. Furthermore, PCNA binding proteins are also important in the context of genotoxic stress [14,15]. The objective of the current study was to investigate the expression levels of CD44, CD90, CD105 and PCNA mRNA in the porcine buccal mucosa cells primary culture on 7th, 15th and 30th day.

Materials and Methods

Animals

A total of 20 pubertal commercially provided crossbred Landrace gilts employed in the study had

a mean age of 155 days (range 140 – 170 days), and the mean weight of 100 kg (95–120 kg). All of the gilts were housed under identical conditions and fed the same forage. All experiments were approved by Local Ethic Committee.

Cell isolation and culture

After slaughter, samples of buccal pouch mucosa were obtained within 40 min and transported to the laboratory. The excised tissue was washed twice in Dulbecco's phosphate buffered saline (D-PBS) (Merck, Darmstadt, Germany). The surface of the buccal pouch was surgically removed using sterile surgical blades. The tissue fragments were incubated with 0.05% collagenase I (Merck, Darmstadt, Germany) for 40 min at 38 °C in a shaking water bath and then were treated with 0.5% Trypsin/EDTA (Cascade Biologics, Portland, USA) for 10 min. The cell suspension obtained from this digestion was filtered through mesh to remove non-dissociated tissue fragments. Isolated cells were washed three times by centrifugation (10 min at 200 g) with Dulbecco's modified Eagle's medium (DMEM; Merck, Darmstadt, Germany) supplemented with gentamicin (20 µg/mL) and 0.1% BSA. The final cell pellet was resuspended in DMEM supplemented with 10% fetal calf serum (FCS; Merck, Darmstadt, Germany) and 10 U/mL penicillin G, 10 mg/mL streptomycin, and 25 µg/mL amphotericin B. Cell viability was 90 to 95% as determined by trypan blue staining (Merck, Darmstadt, Germany). The cells were cultured at 37 °C in a humidified atmosphere of 5% CO₂. Once the cell cultures attained 70–80% confluency, they were passaged by washing with PBS, digested with 0.025% Trypsin/EDTA (Cascade Biologics, Portland, USA), neutralized by a 0.0125% trypsin inhibitor (Cascade Biologics, Portland, USA), centrifuged, and resuspended at a seeding density of 2x10⁴ cells/cm². The culture medium was changed every three days.

Total RNA isolation

After harvesting cells on the 7th, 15th and 30th day of culture, total RNA was isolated. The process of RNA isolation was performed according to modified method of Chomczyński and Sacchi [16]. Briefly, obtained mucosa cells were suspended in 1 ml of monophasic guanidine thiocyanate and phenol solution (TRI Reagent®, Merck KGaA). Next, chloroform was added to separate the phases during centrifugation. The upper aqueous phase, containing isolated RNA, was collected. RNA was extracted with 2-propanol (Merck KGaA, catalog number I9516), added in an amount adequate for 1 ml of TRI-reagent. Finally, RNA was washed with 75% ethanol, dried and resuspended in 20 µl of pure water. Its purity was estimated using the 260/280 nm absorption ratio (higher than 1.8) (NanoDrop spectrophotometer; Thermo Scientific, ALAB, Poland).

Reverse transcription

RNA denaturation involved the preparation of a reaction mixture consisting of 1 µg RNA, 4 µl 2.5 mM dNTP (Novazym, Poland), 0.5 µl oligo (dT) (Novazym, Poland) and 0.5 µl random hexamers (Novazym, Poland). The mixture was then incubated in a thermoblock for 5 min. at 65 °C. The next step was the cDNA synthesis which involved the preparation of a reaction mixture by adding 4 µl of 5x concentrated First Strand Buffer (Invitrogen, USA), 2 µl DTT 0.1 M (Invitrogen, USA), 0.5 µl of SuperScript™ II Reverse Transcriptase reverse transcriptase 200 U / µl (Invitrogen, USA) and 0.25 µl of the ribonuclease inhibitor Rnase OUT 40 U / µl (Invitrogen, USA). The mixture was then incubated in a thermoblock for 50 min. at 37 °C.

Real-Time Quantitative Polymerase Chain Reaction (RT-qPCR) Analysis

1 µL of cDNA obtained from the 7th, 15th and 30th day samples was added to 6 µL pure H₂O, 2 µL Hot Start mastermix QUANTUM EvaGreen® PCR Kit (Syngen Biotech, Wrocław, Poland) and 1 µL of forward and reverse primer mix for amplification. Each sample was repeated in triplicate. One RNA sample of each preparation was processed without the RT-reaction to provide a negative control for subsequent PCR. RT-qPCR was conducted using a Light-Cycler RT-qPCR detection system (Roche Diagnostics GmbH, Mannheim, Germany), with target cDNA was quantified using the relative quantification method.

Ethical approval

The research related to animal use has been complied with all the relevant national regulations and institutional policies for the care and use of animals. Bioethical Committee approval no. 32/2012 from 30.06.2012.

Results

In this research, the relative abundance of CD44, CD90, CD105 and PCNA mRNA in equal time intervals – 7, 15 and 30 days of buccal mucosa cells in vitro cultivation was assessed using the RT-qPCR assay (**Fig. 1**).

The reference value was the expression shown by the CD105 gene on 7th day of culture. In genes CD44, CD90 and PCNA a decrease in expression is seen between days 7 and 30 of cell culture of the buccal mucosa cells. Fold change of CD105 on 7th day of cell culture is a reference value on the figure 1.

In CD44, the highest expression occurs on 7th day of culture, while the lowest on the 30th day of culture. The difference in log(FC) level between 7th and 30th day is 1.43. Expression of this gene measured on 7th day was the highest of all measurements in this study.

CD90 also showed the highest expression on 7th day of culture and the lowest on 30th day. The exponential difference of the first to the last measurement is in this case slightly higher and amounts to 1.81.

The greatest difference between the first and the last measurement is found in the PCNA gene. On day

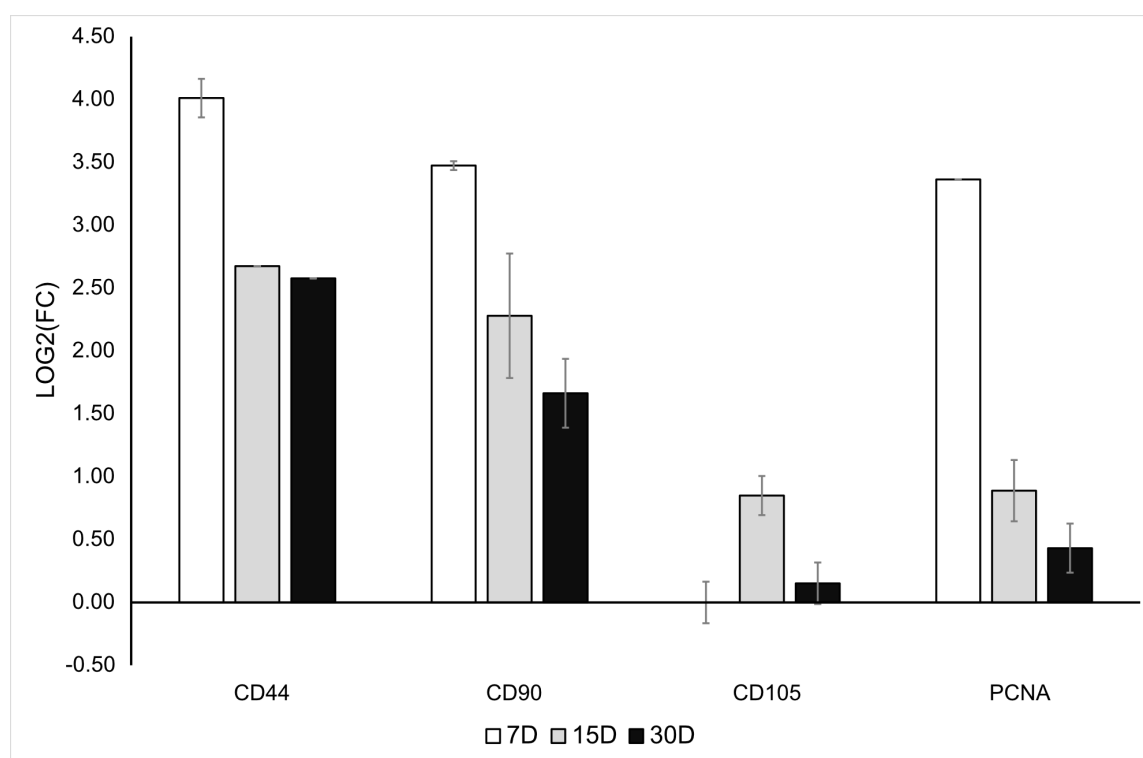


FIGURE 1 Expression of CD44, CD90, CD105 and PCNA mRNA in buccal mucosa cells after various periods of in vitro cultivation; the results are expressed in logarithmic fold change scale

7, FC value was at 3.37, while on 30th it dropped to 0.43. The exponential difference of 7th to 30th day in this case was 2.94.

The highest gene expression for the CD105 gene is at 15th day of cell culture and the lowest on 7th day. Despite the decrease from 15th to 30th day the value was higher than the 7th day level.

Discussion

Discovering, learning and understanding the physiology and genetics of pig as an animal model was undoubtedly one of the breakthrough steps for biomedical research. The transcript level changes (CD44, CD90, CD105, and PCNA) in buccal mucosa cells were examined during the 7th, 15th and 30th day of culture. The CD genes belong to the differentiation clusters which mostly act as ligands or receptors. PCNA, is a DNA clamp necessary for the replication process.

The first gene, CD44, showed the highest expression among all tested genes. Its expression decreased between 7th and 30th day. CD44 is an integral cell membrane glycoprotein, which has a role in cell adhesion to extracellular matrix, lymphocyte activation and lymph node homing [7]. This gene includes 19 exons, covering 50 kb of genomic DNA. 10 alternatively spliced exons have been identified within its extracellular domain. Moreover, according to different studies, this gene is the main cell surface receptor for hyaluronan [17,18]. This chemical compound is important during embryonic development and repair processes. It provides a relatively receptive, highly hydrated matrix through which cells can migrate freely [19]. Epithelial cells showed a high expression of hyaluronan binding activity, and the matrix surrounding them is usually very rich in hyaluronan [29]. CD44 has also documented interactions with mucosal high endothelial venules (HEVs) [17]. Said structures are specialized structures which allow mature lymphocytes migrate in the circulation selectively from the bloodstream to different lymphatic tissues due to homing receptors – molecules on the surface of lymphocytes which interact specifically with HEV [20]. CD44 is also directly related to cancer and cancerogenesis. Splice variants of the CD44 glycoprotein may be useful in the early detection of metastatic potential in surgical biopsies. They can be also useful in the early diagnosis of cancer during screening programs [21,22]. CD44 exhibited very high expression, probably due to the formation and expansion of the ECM. In vitro cultured cells are also able to migrate, and CD44, among others, enables this process.

The CD90 gene ensues next in terms of intensity of expression. As indicated previously, cluster of differentiation 90 also known as CD90 or Thy-1, is a highly N-glycosylated surface protein anchored to glycoposphatidylinositol GPI [23]. This protein is involved in cell communication in several cell ty-

pes, especially in cells of the nervous and immune system. It also plays a significant role in cell adhesion [24,25], cytoskeleton organization [26] and integrin-mediated signaling pathway [27]. Thy-1 was successfully used in research, as a marker for hematopoietic and mesenchymal stem cells [28,29]. Thymus cell antigen 1 was first defined in mice allowing the distinction of T and B lymphocytes from each other [30]. Current studies and analyses of CD90-deficient mice have uncovered its functional impact, being a marker for certain cell types, such as lymphocytes and thymocytes. Thy-1 acts like differentiation-prompted switch in various tissues and controls many signaling pathways [31]. Similar to CD44 gene, CD90 is also involved in adhesion, cytoskeleton organization, and cell-to-cell communication. All these processes take place very intensively at the beginning, when the cells attach to the substrate and then proliferate their numbers - hence the high expression of the CD90 gene.

The last gene to show a pattern of decrease in expression over time is the PCNA gene. Proliferating cell nuclear antigen is a crucial element in DNA replication, encoding the essential accessory protein. Chromatin binding [32], DNA polymerase binding and identical protein binding [33] are just one of the few molecular functions of this gene. The most important biological processes of our interest that requires PCNA, are epithelial cell proliferation and differentiation [34]. Attempts were made to use PCNA in the treatment of atherosclerosis and artery occlusion. Mann et al. (1995) showed that it is possible to use antisense oligodeoxynucleotides in “intraoperative gene therapy” to block the proliferation of smooth medial muscle cells. This may prevent acceleration of atherosclerosis and reduce incidence of complications and ultimately failure in autologous vein graft. A significant finding was that the grafts obtained with this approach proved to be resistant to diet-induced atherosclerosis [35]. Poot et al, while conducting research on Williams syndrome, identified 4 potential PCNA-binding motifs in the transcription factor of this syndrome (WSTF). It has been confirmed that PCNA directs WSTF to DNA replication foci and then WSTF recruits sucrose nonfermenting protein 2 homolog (SNF2H) to replication sites. Increased amount of heterochromatin markers and compaction of newly replicated chromatin were caused by RNA interference-mediated depletion of SNF2H or WSTF [36]. The high expression of PCNA observed in mucosa cells occurred because of the large role that PCNA plays in cell proliferation and differentiation. The lower expression at the end of the culture may be related to the state of cell differentiation.

The only gene in the current study that contradicted the expression pattern of the other 3 genes was CD105. Endoglin (ENG) is other name used to describe cluster of differentiation 105. It is normally found in the vascular endothelium, but it can be also found

in the state of disease in lymphoblasts, monocytes and bone marrow in children with leukemia. This protein is also a component of the transforming growth factor beta (TGFB) receptor complex, where its main role is to bind to TGFB1 with high affinity [11,37,38]. Endoglin is responsible for a multiple biological processes - epithelial to mesenchymal transition [39], cell adhesion [40], cell chemotaxis [41], cell migration [42], cellular response to mechanical stimulus [43], extracellular matrix constituent secretion [44], transforming growth factor beta receptor signaling pathway [45] and wound healing [46] among others. Endoglin expression in normal epithelial cells was investigated by Gurtner et al. showing, that increased expression takes place in mouse epidermal keratinocytes [47]. In tumor studies, it has been found that loss of endoglin expression in epithelial cells is associated with increased metastasis in vivo and in vitro in orthotopic murine prostate cancer models [48]. In breast cancer, expression of endoglin in MDA-MB-231 cells blocked cell motility and prevented TGF- β -enhanced metastasis [49]. Endoglin is also an integral part of MSC research, being, inter alia, their marker [50]. When sorting murine MSCs negative for endoglin, it was found that they inhibit T cell proliferation more effectively than their endoglin-expressing equivalents [51]. The effect of endoglin on fibroblasts was also observed, which after damage show a massive expansion of their population with an activated phenotype [52]. The activated fibroblasts show high TGF- β signaling, which is directly related to PCNA. Fibroblasts begin to produce large amounts of ECM and proteins involved in ECM degradation and remodeling (such as, for example, matrix metalloproteinases (MMPs)) under the influence of TGF- β activation. Thus, the expression of endoglin may contribute to the formation of fibrosis in the tissues, however, there are studies that show its specific protective effect against the formation of fibrosis. It was shown that endoglin deficiency enhanced the expression of fibrotic factors such as fibronectin, so it can be concluded that after correcting the endoglin deficiency, the fibrosis may subside [53].

Conclusions

The CD44, CD90, CD105 and PCNA genes in the current study represent a narrow percentage of the information that should be investigated when exploring knowledge about specific cells or tissues, and the current study provides valuable new baseline information for the model system. The tissues in the oral cavity have a high regenerative potential, which make them suitable model for the research on wound healing processes. The limitations that need to be overcome include different oral microbiome among humans and animals that may have a significant impact on healing processes within the oral cavity of different species.

Acknowledgments

Not applicable.

Corresponding author

Marta Dyszkiewicz-Konwińska, Department of Biomaterials and Experimental Dentistry, 70 Bukowska St., 60-812 Poznań, Poland and Department of Anatomy, Poznań University of Medical Sciences, 6 Święcickiego St., 60-781 Poznań, Poland, Tel./Fax: +48 61 8546565, e-mail: m.dyszkiewicz@ump.edu.pl.

Conflict of interest statement

The authors declare they have no conflict of interest.

References

- Swindle MM, Smith AC. Swine in biomedical research. Source B. Model. Bio-med. Res., Humana Press; 2008; DOI:10.1007/978-1-59745-285-4_26.
- Sullivan TP, Eaglstein WH, Davis SC, Mertz P. The pig as a model for human wound healing. *Wound Repair Regen.* 2001;9:66–76.
- Ross JW, Fernandez de Castro JP, Zhao J, Samuel M, Walters E, Rios C, Bray-Ward P, Jones BW, Marc RE, Wang W, Zhou L, Noel JM, McCall MA, DeMarco PJ, Prather RS, Kaplan HJ. Generation of an inbred miniature pig model of retinitis pigmentosa. *Investig Ophthalmol Vis Sci.* 2012;53:501–7; DOI:10.1167/iops.11-8784.
- Groenen MAM, Archibald AL, Uenishi H, Tuggle CK, Takeuchi Y, Rothschild MF, Rogel-Gaillard C, Park C, Milan D, Megens HJ, Li S, Larkin DM, Kim H, Frantz LAF, Caccamo M, Ahn H, Aken BL, Anselmo A, Anthon C, Auvin L, Schook LB, et al. Analyses of pig genomes provide insight into porcine demography and evolution. *Nature.* 2012;491:393–8; DOI:10.1038/nature11622.
- Zola H, Swart B, Banham A, Barry S, Beare A, Bensussan A, Boumsell L, D. Buckley C, Bühring HJ, Clark G, Engel P, Fox D, Jin BQ, Macardle PJ, Malavasi F, Mason D, Stockinger H, Yang X. CD molecules 2006 - Human cell differentiation molecules. *J Immunol Methods.* 2007;319:1–5; DOI:10.1016/j.jim.2006.11.001.
- Chan JKC, Ng CS, Hui PK. A simple guide to the terminology and application of leucocyte monoclonal antibodies. *Histopathology.* 1988;12:461–80; DOI:10.1111/j.1365-2559.1988.tb01967.x.
- Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronate. *Cell.* 1990;61:1303–13; DOI:10.1016/0092-8674(90)90694-A.
- Morris R. Thy-1 in Developing Nervous Tissue (Part 1 of 2). *Dev Neurosci.* 1985;7:133–46; DOI:10.1159/000112283.
- Boitano AE, Wang J, Romeo R, Bouchez LC, Parker AE, Sutton SE, Walker JR, Flaveny CA, Perdew GH, Denison MS, Schultz PG, Cooke MP. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science (80-).* 2010;329:1345–8; DOI:10.1126/science.1191536.
- Rodríguez-Peña A, Prieto M, Duwel A, Rivas J V., Eleno N, Pérez-Barriocanal F, Arévalo M, Smith JD, Vary CPH, Bernabeu C, López-Novoa JM. Up-regulation of endoglin, a TGF- β -binding protein, in rats with experimental renal fibrosis induced by renal mass reduction. *Nephrol Dial Transplant.* 2001;16:34–9; DOI:10.1093/ndt/16.suppl.1.34.
- Rius C, Smith JD, Almendro N, Langa C, Botella LM, Marchuk DA, Vary CPH, Bernabeu C. Cloning of the Promoter Region of Human Endoglin, the Target Gene for Hereditary Hemorrhagic Telangiectasia Type 1. *Blood.* 1998;92:4677–90; DOI:10.1182/blood.v92.12.4677.
- Bravo R. Synthesis of the nuclear protein cyclin (PCNA) and its relationship with DNA replication. *Exp Cell Res.* 1986;163:287–93; DOI:10.1016/0014-4827(86)90059-5.
- Travali S, Ku DH, Rizzo MG, Ottavio L, Baserga R, Calabretta B. Structure of the human gene for the proliferating cell nuclear antigen. *J Biol Chem.* 1989;264:7466–72.
- Baple EL, Chambers H, Cross HE, Fawcett H, Nakazawa Y, Chioza BA, Harlalka G V., Mansour S, Sreekantan-Nair A, Patton MA, Muggenthaler M, Rich P, Wagner K, Coblenz R, Stein CK, Last JI, Taylor AMR, Jackson AP, Ogi T, Lehmann AR, Green CM, Crosby AH. Hypomorphic PCNA mutation underlies a human DNA repair disorder. *J Clin Invest.* 2014;124:3137–46; DOI:10.1172/JCI74593.
- Mailand N, Gibbs-Seymour I, Bekker-Jensen S. Regulation of PCNA-protein interactions for genome stability. *Nat Rev Mol Cell Biol.* 2013;14:269–82; DOI:10.1038/nrm3562.
- Chomczynski P, Sacchi N. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol-chloroform extraction. *Anal Biochem.* 1987;162:156–9; DOI:10.1016/0003-2697(87)90021-2.
- Culty M, Miyake K, Kincade PW, Silorski E, Butcher EC, Underhill C. The hyaluronate receptor is a member of the CD44 (H-CAM) family of cell surface glycoproteins. *J Cell Biol.* 1990;111:2765–74; DOI:10.1083/jcb.111.6.2765.

18. Misra S, Hascall VC, Markwald RR, Ghatak S. Interactions between hyaluronan and its receptors (CD44, RHAMM) regulate the activities of inflammation and cancer. *Front Immunol.* 2015;6; DOI:10.3389/fimmu.2015.00201.
19. Laurent TC, Fraser JR. The properties and turnover of hyaluronan. *Ciba Found Symp.* 1986;124:9–29; DOI:10.1002/9780470513385.ch2.
20. Clark RA, Alon R, Springer TA. CD44 and hyaluronan-dependent rolling interactions of lymphocytes on tonsillar stroma. *J Cell Biol.* 1996;134:1075–87; DOI:10.1083/jcb.134.4.1075.
21. Basakran NS. CD44 as a potential diagnostic tumor marker. *Saudi Med J.* 2015;36:273–9; DOI:10.15537/smj.2015.3.9622.
22. Matsumura Y, Tarin D. Significance of CD44 gene products for cancer diagnosis and disease evaluation. *Lancet.* 1992;340:1053–8; DOI:10.1016/0140-6736(92)93077-Z.
23. Seki T, Spurr N, Obata F, Goyert S, Goodfellow P, Silver J. The human Thy-1 gene: Structure and chromosomal location. *Proc Natl Acad Sci U S A.* 1985;82:6657–61; DOI:10.1073/pnas.82.19.6657.
24. Killeen N. T-cell regulation: Thy-1 - Hiding in full view. *Curr Biol.* 1997;7:774–7; DOI:10.1016/s0960-9822(06)00402-7.
25. Pont S. Thy-1: a lymphoid cell subset marker capable of delivering an activation signal to mouse T lymphocytes. *Biochimie.* 1987;69:315–20; DOI:10.1016/0300-9084(87)90022-8.
26. Wang Y, Yago T, Zhang N, Abdalsalam S, Alexrakis G, Rodgers W, McEver RP. Cytoskeletal regulation of CD44 membrane organization and interactions with E-selectin. *J Biol Chem.* 2014;289:35159–71; DOI:10.1074/jbc.M114.600767.
27. Leyton L, Díaz J, Martínez S, Palacios E, Pérez LA, Pérez RD. Thy-1/CD90 a Bidirectional and Lateral Signaling Scaffold. *Front Cell Dev Biol.* 2019;7; DOI:10.3389/fcell.2019.00132.
28. Craig W, Kay R, Cutler RL, Lansdorp PM. Expression of Thy-1 on human hematopoietic progenitor cells. *J Exp Med.* 1993;177:1331–42; DOI:10.1084/jem.177.5.1331.
29. An Z, Sabalic M, Bloomquist RE, Fowler TE, Streelman T, Sharpe PT. A quiescent cell population replenishes mesenchymal stem cells to drive accelerated growth in mouse incisors. *Nat Commun.* 2018;9; DOI:10.1038/s41467-017-02785-6.
30. Raff MC. Surface Antigenic Markers for Distinguishing T and B Lymphocytes in Mice. *Immunol Rev.* 1971;6:52–80; DOI:10.1111/j.1600-065X.1971.tb00459.x.
31. Shentu TP, Huang TS, Cernelc-Kohan M, Chan J, Wong SS, Espinoza CR, Tan C, Gramaglia I, Van Der Heyde H, Chien S, Hagood JS. Thy-1 dependent uptake of mesenchymal stem cell-derived extracellular vesicles blocks myofibroblastic differentiation. *Sci Rep.* 2017;7; DOI:10.1038/s41598-017-18288-9.
32. Postnikov Y V, Kurahashi T, Zhou M, Bustin M. The Nucleosome Binding Protein HMGN1 Interacts with PCNA and Facilitates Its Binding to Chromatin. *Mol Cell Biol.* 2012;32:1844–54; DOI:10.1128/mcb.06429-11.
33. González-Magaña A, Blanco FJ. Human PCNA structure, function and interactions. *Biomolecules.* 2020;10; DOI:10.3390/biom10040570.
34. Sourisseau T, Georgiadis A, Tsapara A, Ali RR, Pestell R, Matter K, Balda MS. Regulation of PCNA and Cyclin D1 Expression and Epithelial Morphogenesis by the ZO-1-Regulated Transcription Factor ZONAB/DbpA. *Mol Cell Biol.* 2006;26:2387–98; DOI:10.1128/mcb.26.6.2387-2398.2006.
35. Mann MJ, Gibbons GH, Kernoff RS, Diet FP, Tsao PS, Cooke JP, Kaneda Y, Dzau VJ. Genetic engineering of vein grafts resistant to atherosclerosis. *Proc Natl Acad Sci U S A.* 1995;92:4502–6; DOI:10.1073/pnas.92.10.4502.
36. Poot RA, Bozhenok L, van den Berg DLC, Steffensen S, Ferreira F, Grimaldi M, Gilbert N, Ferreira J, Varga-Weisz PD. The Williams syndrome transcription factor interacts with PCNA to target chromatin remodelling by ISWI to replication foci. *Nat Cell Biol.* 2004;6:1236–44; DOI:10.1038/ncb1196.
37. Dourado KMC, Baik J, Oliveira VKP, Beltrame M, Yamamoto A, Theuer CP, Figueiredo CAV, Verneris MR, Perlingeiro RCR. Endoglin: A novel target for therapeutic intervention in acute leukemias revealed in xenograft mouse models. *Blood.* 2017;129:2526–36; DOI:10.1182/blood-2017-01-763581.
38. Rossi E, Smadja DM, Boscolo E, Langa C, Arevalo MA, Pericacho M, Gamella-Pozuelo L, Kauskot A, Botella LM, Gaussem P, Bischoff J, Lopez-Novoa JM, Bernabeu C. Endoglin regulates mural cell adhesion in the circulatory system. *Cell Mol Life Sci.* 2016;73:1715–39; DOI:10.1007/s00018-015-2099-4.
39. Mitselou A, Galani V, Skoufi U, Arvanitis DL, Lampri E, Ioachim E. Syndecan-1, epithelial-mesenchymal transition markers (E-cadherin/ β -catenin) and neoangiogenesis-related proteins (PCAM-1 and Endoglin) in colorectal cancer. *Anticancer Res.* 2016;36:2271–80.
40. Rossi E, Lopez-Novoa JM, Bernabeu C. Endoglin involvement 1 in integrin-mediated cell adhesion as a putative pathogenic mechanism in Hereditary Hemorrhagic Telangiectasia type 1 (HHT1). *Front Genet.* 2014;5; DOI:10.3389/fgene.2014.00457.
41. Rossi E, Sanz-Rodriguez F, Eleno N, Düwell A, Blanco FJ, Langa C, Botella LM, Cabañas C, Lopez-Novoa JM, Bernabeu C. Endothelial endoglin is involved in inflammation: Role in leukocyte adhesion and transmigration. *Blood.* 2013;121:403–15; DOI:10.1182/blood-2012-06-435347.
42. Tian H, Ketova T, Hardy D, Xu X, Gao X, Zijlstra A, Blobel GC. Endoglin Mediates Vascular Maturation by Promoting Vascular Smooth Muscle Cell Migration and Spreading. *Arterioscler Thromb Vasc Biol.* 2017;37:1115–26; DOI:10.1161/ATVBAHA.116.308859.
43. Maul TM, Chew DW, Nieponice A, Vorp DA. Mechanical stimuli differentially control stem cell behavior: morphology, proliferation, and differentiation. *Biomech Model Mechanobiol.* 2011;10:939–53; DOI:10.1007/s10237-010-0285-8.
44. Díez-Marques L, Ortega-Velazquez R, Langa C, Rodríguez-Barbero A, Lopez-Novoa JM, Lamas S, Bernabeu C. Expression of endoglin in human mesangial cells: Modulation of extracellular matrix synthesis. *Biochim Biophys Acta - Mol Basis Dis.* 2002;1587:36–44; DOI:10.1016/S0925-4439(02)00051-0.
45. Lee NY, Ray B, How T, Blobel GC. Endoglin promotes transforming growth factor β -mediated Smad 1/5/8 signaling and inhibits endothelial cell migration through its association with GIPC. *J Biol Chem.* 2008;283:32527–33; DOI:10.1074/jbc.M803059200.
46. Pérez-Gómez E, Jerkic M, Prieto M, Del Castillo G, Martín-Villar E, Letarte M, Bernabeu C, Pérez-Barriocanal F, Quintanilla M, López-Novoa JM. Impaired wound repair in adult endoglin heterozygous mice associated with lower NO bioavailability. *J Invest Dermatol.* 2014;134:247–55; DOI:10.1038/jid.2013.263.
47. Gurtner GC, Werner S, Barrandon Y, Longaker MT. Wound repair and regeneration. *Nature.* 2008;453:314–21; DOI:10.1038/nature07039.
48. Lakshman M, Huang X, Ananthanarayanan V, Jovanovic B, Liu Y, Craft CS, Romero D, Vary CPH, Bergan RC. Endoglin suppresses human prostate cancer metastasis. *Clin Exp Metastasis.* 2011;28:39–53; DOI:10.1007/s10585-010-9356-6.
49. Henry LA, Johnson DA, Sarrió D, Lee S, Quinlan PR, Crook T, Thompson AM, Reis-Filho JS, Isacke CM. Endoglin expression in breast tumor cells suppresses invasion and metastasis and correlates with improved clinical outcome. *Oncogene.* 2011;30:1046–58; DOI:10.1038/ncr.2010.488.
50. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM. Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. *Cytotherapy.* 2006;8:315–7; DOI:10.1080/14653240600855905.
51. Anderson P, Carrillo-Gálvez AB, García-Pérez A, Cobo M, Martín F. CD105 (Endoglin)-Negative Murine Mesenchymal Stromal Cells Define a New Multipotent Subpopulation with Distinct Differentiation and Immunomodulatory Capacities. *PLoS One.* 2013;8; DOI:10.1371/journal.pone.0076979.
52. Velnar T, Bailey T, Smrkolj V. The wound healing process: An overview of the cellular and molecular mechanisms. *J Int Med Res.* 2009;37:1528–42; DOI:10.1177/147323000903700531.
53. Alsamman M, Sterzer V, Meurer SK, Sahin H, Schaeper U, Kuscuglu D, Strnad P, Weiskirchen R, Trautwein C, Scholten D. Endoglin in human liver disease and murine models of liver fibrosis—A protective factor against liver fibrosis. *Liver Int.* 2018;38:858–67; DOI:10.1111/liv.13595.