

- Kumar S., Pan C.C., Bloodworth J.C., Nixon A.B., Theuer C., Hoyt D.G., Lee N.Y. 2014. Antibody-directed coupling of endoglin and MMP-14 is a key mechanism for endoglin shedding and deregulation of TGF- β signaling. *Oncogene*. V. 33. P. 3970.
- Liu Y., Tian H., Blobe G.C., Theuer C.P., Hurwitz H.I., Nixon A.B. 2014. Effects of the combination of TRC105 and bevacizumab on endothelial cell biology. *Invest. New Drugs*. V. 32. P. 851.
- López-Novoa J.M., Bernabeu C. 2010. The physiological role of endoglin in the cardiovascular system. *Am. J. Physiol. Heart Circ. Physiol.* V. 299. P. H959.
- Mutin M., Dignat-George F., Sampol J. 1997. Immunologic phenotype of cultured endothelial cells: quantitative analysis of cell surface molecules. *Tissue Antigens*. V. 50. P. 449.
- Nassiri S.M., Rahbarghazi R. 2014. Interactions of mesenchymal stem cells with endothelial cells. *Stem Cells Dev.* V. 23. P. 319.
- Nolan-Stevaux O., Zhong W., Culp S., Shaffer K., Hoover J., Wickramasinghe D., Ruefli-Brasse A. 2012. Endoglin requirement for BMP9 signaling in endothelial cells reveals new mechanism of action for selective anti-endoglin antibodies. *PLoS One*. V. 7. P. e50920.
- Quinn G., Keough M. 2002. Experimental design and data analysis for biologists. Cambridge University Press. 557 pp.
- Rokhlin O.W., Cohen M.B., Kubagawa H., Letarte M., Coope M.D. 1995. Differential expression of endoglin on fetal and adult hematopoietic cells in human bone marrow. *J. Immunol.* V. 154. P. 4456.
- Rosen L.S., Gordon M.S., Robert F., Matei D.E. 2014. Endoglin for targeted cancer treatment. *Curr. Oncol. Rep.* V. 16. P. 365.
- Rossi E., López-Novoa J.M., Bernabeu C. 2015. Endoglin involvement in integrin-mediated cell adhesion as a putative pathogenic mechanism in hereditary hemorrhagic telangiectasia type 1 (HHT1). *Front. Genet.* V. 5. P. 1.
- Rossi E., Pericacho M., Bachelot-Loza C., Pidard D., Gaussem P., Poirault-Chassac S., Blanco F.J., Langa C., González-Manchyn C., López-Novoa J.M., Smadja D.M., Bernabeu C. 2018. Human endoglin as a potential new partner involved in platelet-endothelium interactions. *Cell. Mol. Life Sci.* V. 75. P. 1269.
- Sánchez-Elsner T., Botella L.M., Velasco B., Langa C., Bernabeu C. 2002. Endoglin expression is regulated by transcriptional cooperation between the hypoxia and transforming growth factor- β pathways. *J. Biol. Chem.* V. 277. P. 43799.
- Sanz-Rodríguez F., Guerrero-Esteo M., Botella L.M., Banville D., Vary C.P.H., Bernabeu C. 2004. Endoglin regulates cytoskeletal organization through binding to ZRP-1, a member of the Lim family of proteins. *J. Biol. Chem.* V. 279. P. 32858.
- Sugden W.W., Meissner R., Aegerter-Wilmsen T., Tsaryk R., Leonard E.V., Bussmann J., Hamm M.J., Herzog W., Jin Y., Jakobsson L., Denz C., Siekmann A.F. 2017. Endoglin controls blood vessel diameter through endothelial cell shape changes in response to haemodynamic cues. *Nat. Cell Biol.* V. 19. P. 653.
- Tian H., Ketova T., Hardy D., Xu X., Gao X., Zijlstra A., Blobe G.C. 2017. Endoglin mediates vascular maturation by promoting vascular smooth muscle cell migration and spreading. *Arterioscler. Thromb. Vasc. Biol.* V. 37. P. 1115.
- Tian H., Mythreya K., Golzio C., Katsanis N., Blobe G.C. 2012. Endoglin mediates fibronectin/ $\alpha 5 \beta 1$ integrin and TGF- β pathway crosstalk in endothelial cells. *EMBO J.* V. 31. P. 3885.
- Tian F., Zhou A.X., Smits A.M., Larsson E., Goumans M.J., Heldin C.H., Borén J., Akyürek L.M. 2010. Endothelial cells are activated during hypoxia via endoglin/ALK-1/SMAD1/5 signaling *in vivo* and *in vitro*. *Biochem. Biophys. Res. Commun.* V. 392. P. 283.
- Unger R.E., Krump-Konvalinkova V., Peters K., Kirkpatrick J.C. 2002. *In vitro* expression of the endothelial phenotype: comparative study of primary isolated cells and cell lines, including the novel cell line HPMEC-ST1.6R. *Microvasc. Res.* V. 64. P. 384.
- Warrington K., Hillarby M.C., Li C., Letarte M., Kumar S. 2005. Functional role of CD105 in TGF β -1 signalling in murine and human endothelial cells. *Anticancer Res.* V. 25. P. 1851.
- Wickham H. 2016. ggplot2: elegant graphics for data analysis. 253 pp.
- Zuur A.F., Ieno E.N., Walker N., Saveliev A.A., Smith G.M. 2009. Mixed effects models and extensions in ecology with R. Springer. 574 pp.

Comparative Analysis of Endoglin Antibodies Effect on the Functional Characteristics of Endothelial Cells HUVEC and EA.hy926

A. Y. Stolbovaya^{a, b, *}, I. V. Smirnov^a, A. A. Pinevich^{a, c}, N. L. Vartanyan^a, I. Y. Krutetskaya^a, L. A. Terekhina^a, K. L. Markova^d, A. B. Malashicheva^b, and M. P. Samoilovich^{a, c}

^a Granov Russian Research Center for Radiology and Surgical Technologies, St. Petersburg, 197758 Russia

^b Almazov National Medical Research Center, St. Petersburg, 197341 Russia

^c Saint Petersburg State University, Department of Cytology and Histology, Saint Petersburg, 199034 Russia

^d The Ott Research Institute of Obstetrics, Gynecology and Reproductology, Saint Petersburg, 199034 Russia

*e-mail: anastasia.stolbovaya@gmail.com

Endoglin, a co-receptor of TGF β -family growth factors, is a marker of endothelial cells. In our previous work we have demonstrated that monoclonal antibodies (MABs) against endoglin can change the functional properties of en-

dothelial cells EA.hy926. The aim of the present work was to study the effect of the same antibodies against endoglin on HUVEC endothelial cells, as well as to compare the data obtained for HUVEC and EA.hy926 cells. Comparison of *in vitro* models based on the permanent EA.hy926 endothelial cells and primary HUVEC cells revealed, along with common morpho-functional properties, a number of dissimilarities in adhesion molecules expression level and endothelial differentiation genes activity. Anti-endoglin MABs 2C8 and 4E4 were shown to inhibit HUVEC cell migration, reduce their adhesion to solid substrate, alter the arrangement of actin microfilaments, and impede the formation of capillary-like structures. These effects were revealed only in the presence of TGF- β 1 in the culture medium or under hypoxia. Supplementation of the growth medium with MAB 2C8 promoted endoglin shedding from HUVEC cells membrane both in hypoxia and normoxia. Several impacts of anti-endoglin MABs on HUVEC cultures were similar to those detected on EA.hy926 cells. However MAB 2C8 influenced endoglin shedding oppositely in HUVEC and EA.hy926 cells.

Keywords: endoglin, endothelial cells, EA.hy926, HUVEC, monoclonal antibodies