Scientific report for the period of 2012. 09.01-2015.08.31.

Principal investigator: **Beata Lontay PhD, University of Debrecen, Department of Medical Chemistry**

The official report of the grant #OTKA PD 104878 entiteled "**Regulation of muscle plasticity by the smoothelin-like protein 1**" for the period of 2013.09.01-2015.08.31 is as follows.

Relatively little information exists on the mechanism of smooth muscle adaptation during pregnancy. Because myosin phosphatase (MP) is the primary effector of smooth muscle relaxation and key target of signalling pathways regulating vascular tone, we hypothesize that the expression of myosin phosphatase's regulatory subunit (MYPT1) would be altered in these conditions and it might be regulated by its potential effector SMTNL1. Our goal is to carry out experiments to describe the molecular interaction between SMTNL1 and MYPT and the regulation of their protein-protein interaction through PKA/PKG signalling. We intend to determine the repressor mechanism by which SMTNL1 acts on mypt expression through steroid hormone receptors. Our aim is also to investigate whether SMTNL1 is a selective cofactor of mypt or can act on other genes coding contractile and metabolic proteins or cytokines regulating smooth muscle functions. Hereby we hypothesize a new mechanism for muscle adaptation as a response to physiological changes. The mechanism reveals the regulatory role of SMTNL1 through MP. In our understanding SMTNL1 modulates MP by a dual mechanism in muscle adaptation. Under normal conditions, SMTNL1 modulates MP activity by molecular interaction regulated through PKA/PKG signaling, while upon physiological stress such as pregnancy it regulates the expression of MYPT as a cofactor at the transcriptional level through progesterone receptor.

Results of Hypothesis 1:

Our primary aim was to investigate the protein-protein interaction between SMTNL1 and MYPT and their localization depending on their phosphorylation state. SMTNL1 was shown to have an inhibitory effect on MP activity towards phosphorylated MLC_{20} substrate *in vitro*. Studies in permeabilized smooth muscle suggested a role in mediating Ca^{2+} desensitization in response to cGMP and the deletion of SMTNL1 caused a significant decrease in contractile force. Moreover, while full-length SMTNL1 could suppress MP activity toward MLC_{20} *in vitro*, truncated SMTNL1 lacking the calponin homology domain

was ineffective suggesting the interaction of the N-terminal SMTNL1 at a so far unknown specific site of MYPT. SMTNL1 and MYPT may interact and their protein-protein interaction and localization depend on their phosphorylation state: As we reported before, using immunoprecipitation, Surface Plasmon resonance-based technology we have investigated the protein-protein interaction between SMTNL1 and MYPT and their localization depending on

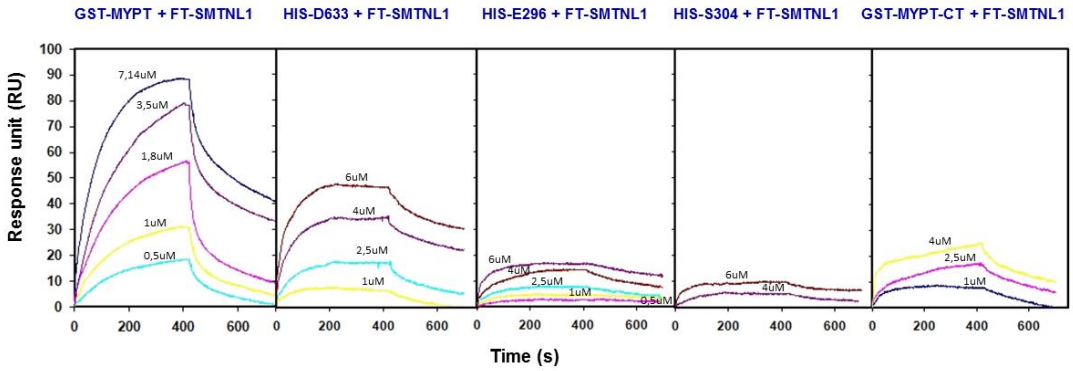

Figure 1. *SMTNL1 interacts with the 1-296 region of the GST-MYPT by Surface Plasmon resonance measurements applying recombinant truncated forms of GST-MYPT in the sensor chip.*

their phosphorylation state. We have described the interaction between MYPT and SMTNL1. These results suggest that the region of the SMTNL1 binding site on MYPT is the N-terminal MYPT1-296 region (Figure 1.). The phosphorylation of SMTNL1 and MYPT1 and their interaction upon posttranslational modification have been characterized.

Results of Hypothesis 2:

The other aim was to study the effect of pregnancy on smooth and skeletal muscle and the role of SMTNL1 in this process. Pregnancy promotes physiological adaptations throughout the body, mediated by the female sex hormones progesterone and estrogen. During pregnancy, changes in the metabolic properties of skeletal muscle may be responsible for the development of insulin resistance. We conducted global microarray, proteomic and metabolic analyses to study the role of the progesterone receptor (PR) and its transcriptional regulator, smoothelin like protein 1 (SMTNL1) in the adaptation of skeletal muscle to pregnancy. We demonstrate that pregnancy promotes fiber type changes from an oxidative to glycolytic isotype in skeletal muscle. This phenomenon is regulated through an interaction between SMTNL1 and PR, which alters the expression of contractile and metabolic proteins. *Smtnl1-/-* mice are metabolically less efficient and show impaired glucose tolerance.

Pregnancy antagonizes these effects by inducing metabolic activity, reducing oxygen consumption and increasing glucose tolerance. Our results suggest that SMTNL1 has a role in mediating the actions of steroid hormones to promote fiber-switching in skeletal muscle during pregnancy (Figure 2.). These results have been analysed and summarized in a publication.

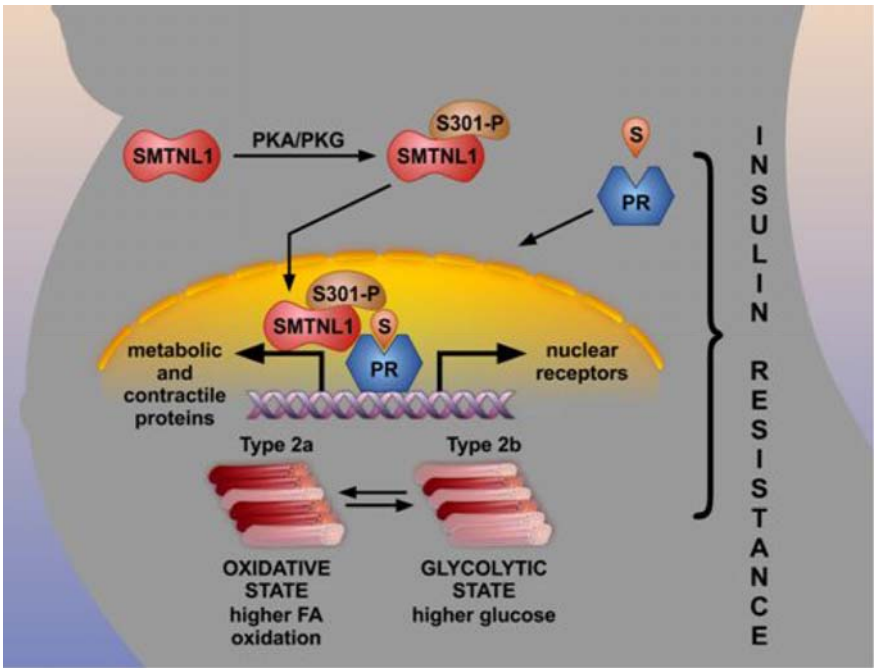

Figure 2. Schematic showing the molecular mechanism by which SMTNL1 regulates PR-B to co-ordinately promote skeletal muscle adaptations in response to pregnancy*. In response to elevated steroid hormone, progesterone (S) levels SMTNL1 and PR-B (R) enter the nucleus. In the non-pregnant state SMTNL1 functions to repress PR-B activity however, during pregnancy this inhibition is relieved to promoting activation of skeletal muscle specific genes regulating the expression of metabolic and contractile proteins. This coordinated response adapts the mothers physiological state to support the weight gain originating from developing fetus. Switching to a glycolytic phenotype in skeletal muscle reduces the oxidative capacity of SKM promoting increased storage of fatty acid (FA) and inducing an insulin resistant state resulting in increased circulating glucose.*

Results of Hypothesis 3:

Bioinformatics analysis of data from global gene expression and multidimensional proteomic analyses showed that pregnancy highly regulates sets of genes related to canonical pathways that play a role in glycolysis, gluconeogenesis, muscle contraction, steroid biosynthesis as well as inflammatory responses, supporting our hypothesis of the manifestation of a glycolytic phenotype in pregnant SKM. The main profile of genes induced in *smtnl1-/-* deletion was transcriptional regulation, suggesting a primary role of SMTNL1 in

gene expression since genes such as PR and nuclear receptor subfamily 3-4 are regulated. SMTNL1 promotes alterations as a cofactor on the transription of genes related to muscle adaptation: SMTNL1 may play a role as a transcriptional cofactor not only selectively for *mypt* but may regulate the expression of cytokines such as interleukin-6 (IL-6). The secreted IL-6 concentration of *smtnl1-/-* mice by ELISA, Luminex and Q-PCR were significantly higher compared to WT littermates and muscle from KO mice presented stronger IL-6 staining by immunohistochemical assay Figure 3.). Although the mechanism is still not clear

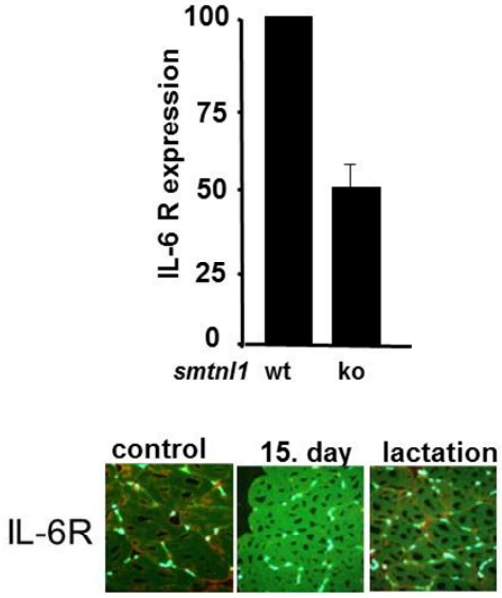

Figure 3. *SMTNL1 interacts with the 1-296 region of the GST-MYPT.*

but IL-6 and inflammatory factors play a role in the pathogenesis of type 1 and 2 diabates, and it also could be regulated through PKA/PKG signalling pathways. IL-6 affect the homeostasis and metabolism in the muscle and the mediation of its expression can lead to decreased the manifestation of diabetes. We screened if SMTNL1 has a general regulatory role in muscle cells. We continue in the last year of the grant our experiments on SMTNL1-silenced L6.G8. skeletal muscle cell line using lentiviral system to assay the cytokine concentration by ELISA and also to presented the metabolic changes of these cells upon pregnancy hormones. The completition of the last subprojects requires few more months.

During the period of the grant 7 posters and one invited talk at international, while 8 posters and two invited national talks have been presented by the support of OTKA. Four BSc and two MSc dissertations have been completed and one PhD student passed successfuly the

doctoral comprehensive exam and getting ready to defense her thesis on this topic. Four papers were supported by the project and have been published in peer-reviewed papers:

Lontay B, Bodoor K, Sipos A, Weitzel DH, Loiselle D, Safi R, Zheng D, Devente J, Hickner RC, McDonnell DP, Ribar T, Haystead TA. J Biol Chem. 2015 Jul 17;290(29):17985-98. doi: 10.1074/jbc.M115.658120. Epub 2015 Jun 5. *Pregnancy and Smoothelin-like Protein 1 (SMTNL1) Deletion Promote the Switching of Skeletal Muscle to a Glycolytic Phenotype in Human and Mice.*

Dedinszki D, Sipos A, Kiss A, Bátori R, Kónya Z, Virág L, Erdődi F, **Lontay B,** Biochim Biophys Acta. 2015 Jan;1852(1):22-33. doi: 10.1016/j.bbadis.2014.11.005. Epub 2014 Nov 8. *Protein phosphatase-1 is involved in the maintenance of normal homeostasis and in UVA irradiation-induced pathological alterations in HaCaT cells and in mouse skin.*

Bécsi B[1], Dedinszki D[2], Gyémánt G[3], Máthé C[4], Vasas G[4]**, Lontay B**[2], Erdődi F[5].
J Photochem Photobiol B. 2014 Sep 5;138:240-8. doi: 10.1016/j.jphotobiol.2014.06.004. Epub 2014 Jun 14. *Identification of protein phosphatase interacting proteins from normal and UVA-irradiated HaCaT cell lysates by surface plasmon resonance based binding technique using biotin-microcystin-LR as phosphatase capturing molecule.*

Ruzsnavszky O, Dienes B, Oláh T, Vincze J, Gáll T, Balogh E, Nagy G, Bátori R, **Lontay B**, Erdődi F, Csernoch L. *Differential effects of phosphatase inhibitors on the calcium homeostasis and migration of HaCaT keratinocytes.* PLoS One. 2013 Apr 30;8(4):e61507. doi: 10.1371/journal.pone.0061507. Print 2013.

Manuscripts are in preparation: one paper is ready for the pre-submission and one manuscript is in the preparatory phase:

Sipos A[1], Darula Zs[4], Horváth D.[1], Dienes B[2], Bécsi B[1, 3], Erdődi F[1, 3]**, Lontay B**[1] *Myosin phosphatase and Rho A activated kinase modulate arginine methylation by the regulation of PRMT5*.

Horváth D, Tamás I, Sipos A, Pál B[2]; Erdődi F[1, 3] **Lontay B**[1]; *The role of myosin phosphatase and Rho-a activated kinase in the regulation of neurotransmitter release through the regulation of SNARE complex*.