TAP2 EFFECT ON MIN-PIG STROMAL VASCULAR FRACTION CELL GENE EXPRESSION

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We have hypothesized that the TAP2 gene is associated with lipid metabolism. Here, 10 Min-pig tissues were collected to detect the expression of TAP2 in different tissues. We obtained dorsal subcutaneous structural vascular fraction (SVF) cells from the Min-pig's back adipose tissue and induced SVF cells into mature adipocytes. By overexpression and interference, the effect of TAP2 on fat deposition in Min-pig SVF cells was studied. Recombinant human insulin, dexamethasone, indomethacin, 3-isobutyl-1-methylxanthine, triiodothyronine, and rosiglitazone could successfully induce SVF cells into mature adipocytes, and the induction efficiency was above 50 %. The tissue expression profile showed that TAP2 was expressed in different tissues, and the highest expression was found in back fat, spleen, and back muscle. Overexpression of the TAP2 gene in SVF cells could significantly increase the expression of adipose differentiation-related genes. The expression of TAP2 in SVF cells was reduced to 0.6 times after transfection of the TAP2 gene interference fragment. The adipogenic transcription factor gene C/EBPα, fatty acid synthase gene FSA, and adipocyte directional differentiation factor ADD1 were downregulated, while the expression of lipolysis-related gene LPL was inhibited. In conclusion, TAP2 expression can promote the deposition of subcutaneous fat on Min-pig's back adipose tissue.

Key words: *gene interference, gene overexpression, Min-pig, SVF cells, TAP2.*

ВПЛИВ *TAP2* НА ЕКСПРЕСІЮ ГЕНІВ КЛІТИН СТРОМАЛЬНОЇ СУДИННОЇ ФРАКЦІЇ У СВИНЕЙ ПОРОДИ МІН

Ми припустили, що ген *TAP2* пов'язаний з метаболізмом ліпідів. Для виявлення експресії *TAP2* різних тканин використали 10 зразків тканин, отриманих від свиней породи Мін. Ми отримали клітини дорсальної підшкірної стромальної судинної фракції (ССФ) із хребтових жирових тканин свиней породи Мін та індукували клітини ССФ у зрілі жирові клітини. Вплив TAP2 на відкладення жиру в ССФ клітинах свиней породи Мін вивчали за надмірною експресією та інтерференцією. Рекомбінантний інсулін людини, дексаметазон, індометацин, 3-ізобутил-1-метилксантин, трийодтиронін і розиглітазон були здатні успішно індукувати ССФ клітини в зрілі жирові клітини; ефективність індукції становила більше 50 %. Профіль експресії тканин продемонстрував, що експресія *TAP2* проходила в різних тканинах, найвищий рівень експресії було зафіксовано в хребтовому жиру, селезінці та спинних м'язах. Надмірна експресія гену *TAP2* у ССФ клітинах може значно підвищити рівень експресії генів жирових клітин, пов'язаних із диференціацією. Експресія *TAP2* у ССФ клітинах знизилась у 0,6 рази після трансфекції фрагменту інтерференції гену *TAP2.* Було відмічено негативне регулювання адипогенного гена фактора транскрипції *C/EBPα*, гена синтази жирних кислот *FSA* та адипоцитного фактора диференціації спрямованої дії *ADD1,* а також інгібування експресії гена *LPL*, пов'язаного з ліполізом. Отже, експресія *TAP2* може сприяти відкладенню підшкірного жиру в хребтових жирових тканинах свиней породи Мін.

Ключові слова: інтерференція гена, надмірна експресія гена, свині породи Мін, клітини ССФ, TAP2.

REFERENCES

Al-Ghadban S, Diaz ZT, Singer HJ et al (2020) Increase in Leptin and PPAR-gamma Gene Expression in Lipedema Adipocytes Differentiated *in vitro* from Adipose-Derived Stem Cells Cells 9(2). https://doi.org/10.3390/cells9020430

Albuquerque A, Ovilo C, Nunez Y et al (2021) Transcriptomic Profiling of Skeletal Muscle Reveals Candidate Genes Influencing Muscle Growth and Associated Lipid Composition in Portuguese Local Pig Breeds Animals (Basel) 11(5). https://doi.org/10.3390/ani11051423

Alharthi AM, Banaganapalli B, Hassan SM et al (2022) Complex Inheritance of Rare Missense Variants in PAK2, TAP2, and PLCL1 Genes in a Consanguineous Arab Family With Multiple Autoimmune Diseases Including Celiac Disease. Front Pediatr 10:895298. https://doi.org/10.3389/fped.2022.895298

Barr VA, Balagopalan L, Barda-Saad M et al (2006) T-cell antigen receptor-induced signaling complexes: internalization via a cholesterol-dependent endocytic pathway. Traffic 7(9):1143–1162. https://doi.org/10.1111/j.1600-0854.2006.00464.x

Belleville-Rolland T, Leuci A, Mansour A et al (2021) Role of membrane lipid rafts in MRP4 (ABCC4)-dependent regulation of the cAMP pathway in blood platelets. Thromb Haemost. https://doi.org/10.1055/a-1481-2663

Catani MV, Tullio V, Maccarrone M et al (2023) DNA-Protein-Interaction (DPI)-ELISA Assay for PPAR-gamma Receptor Binding. Methods Mol Biol 2576:133–143. https://doi.org/10.1007/978-1-0716-2728-0_10

Chen J, Zhang H, Gao H et al (2019) Effects of Dietary Supplementation of Alpha-Ketoglutarate in a Low-Protein Diet on Fatty Acid Composition and Lipid Metabolism Related Gene Expression in Muscles of Growing Pigs. Animals (Basel) 9(10). https://doi.org/10.3390/ani9100838

Chen Z, Torrens JI, Anand A et al (2005) Krox20 stimulates adipogenesis via C/EBPbeta-dependent and -independent mechanisms. Cell Metab 1(2):93–106. https://doi.org/10.1016/j.cmet.2004.12.009

Cimmino I, Oriente F, D'Esposito V et al (2019) Low-dose Bisphenol-A regulates inflammatory cytokines through GPR30 in mammary adipose cells. J Mol Endocrinol 63(4):273–283. https://doi.org/10.1530/JME-18-0265

Darazam IA, Hakamifard A, Momenilandi M et al (2023) Delayed Diagnosis of Chronic Necrotizing Granulomatous Skin Lesions due to TAP2 Deficiency. J Clin Immunol 43(1):217–228. https://doi.org/10.1007/s10875-022-01374-7

El-Assaad A, Dawy Z, and Nemer G (2015) Electrostatic study of Alanine mutational effects on transcription: application to GATA-3:DNA interaction complex. Annu Int Conf IEEE Eng Med Biol Soc 2015:4005–4008. https://doi.org/10.1109/EMBC.2015.7319272

Farmer SR (2006) Transcriptional control of adipocyte formation. Cell Metab 4(4):263–273. https://doi.org/10.1016/j.cmet.2006.07.001

Feng Y, An Z, Chen H et al (2020) Ulva prolifera Extract Alleviates Intestinal Oxidative Stress via Nrf2 Signaling in Weaned Piglets Challenged With Hydrogen Peroxide. Front Immunol 11:599735. https://doi.org/10.3389/fimmu.2020.599735

Flori L, Rogel-Gaillard C, Cochet M et al (2008) Transcriptomic analysis of the dialogue between Pseudorabies virus and porcine epithelial cells during infection. BMC Genomics 9:123. https://doi.org/10.1186/1471-2164-9-123

Gajate C, and Mollinedo F (2021) Lipid Raft Isolation by Sucrose Gradient Centrifugation and Visualization of Raft-Located Proteins by Fluorescence Microscopy: The Use of Combined Techniques to Assess Fas/CD95 Location in Rafts During Apoptosis Triggering. Methods Mol Biol 2187:147–186. https://doi.org/10.1007/978-1-0716-0814-2_9

Garcia-Borges CN, Phanavanh B, and Crew MD (2006) Characterization of porcine TAP genes: alternative splicing of TAP1. Immunogenetics 58(5–6):374–382. https://doi.org/10.1007/s00251-006-0103-8

Han SL, Liu Y, Limbu SM et al (2021) The reduction of lipid-sourced energy production caused by ATGL inhibition cannot be compensated by activation of HSL, autophagy, and utilization of other nutrients in fish. Fish Physiol Biochem 47(1):173–188. https://doi.org/10.1007/s10695-020-00904-7

Huang W, Zhang X, Li A et al (2018) Genome-Wide Analysis of mRNAs and lncRNAs of Intramuscular Fat Related to Lipid Metabolism in Two Pig Breeds. Cell Physiol Biochem 50(6):2406–2422. https://doi.org/10.1159/000495101

Jiang MJ, Li L, Huang WF et al (2021) Rubus chingii var. suavissimus alleviates high-fat diet-induced lipid metabolism disorder via modulation of the PPARs/SREBP pathway in Syrian golden hamsters. J Nat Med. https://doi.org/10.1007/s11418-021-01536-8

Lee SH, Lee SH, Park HB et al (2023) Identification of key adipogenic transcription factors for the pork belly parameters via the association weight matrix. Meat Sci 195:109015. https://doi.org/10.1016/j.meatsci.2022.109015

Li D, Zhang F, Zhang X et al (2016) Distinct functions of PPARgamma isoforms in regulating adipocyte plasticity. Biochem Biophys Res Commun 481 (1–2):132–138. https://doi.org/10.1016/j.bbrc.2016.10.152

Lin J, Cao C, Tao C et al (2017) Cold adaptation in pigs depends on UCP3 in beige adipocytes. J Mol Cell Biol 9(5):364–375. https://doi.org/10.1093/jmcb/mjx018

Liu L, Cao P, Zhang L et al (2021) Comparisons of adipogenesis- and lipid metabolism-related gene expression levels in muscle, adipose tissue and liver from Wagyu-cross and Holstein steers. PLoS One 16(2):e0247559. https://doi.org/10.1371/journal.pone.0247559

Liu Y, Yang X, Jing X et al (2017) Transcriptomics Analysis on Excellent Meat Quality Traits of Skeletal Muscles of the Chinese Indigenous Min Pig Compared with the Large White Breed. Int J Mol Sci 19(1). https://doi.org/10.3390/ijms19010021

Ma W, Lehner PJ, Cresswell P et al (1997) Interferon-gamma rapidly increases peptide transporter (TAP) subunit expression and peptide transport capacity in endothelial cells. J Biol Chem 272(26):16585–16590. https://doi.org/10.1074/jbc.272.26.16585

Nagy N, Vanky F, and Klein E (1998) Tumor surveillance: expression of the transporter associated with antigen processing (TAP-1) in *ex vivo* human tumor samples and its elevation by *in vitro* treatment with IFN-gamma and TNF-alpha. Immunol Lett 64 (2–3):153–160. https://doi.org/10.1016/s0165-2478(98)00104-7

Nian HY, Zhang RX, Ding SS et al (2023) Emotional responses of piglets under long-term exposure to negative and positive auditory stimuli. Domest Anim Endocrinol 82:106771. https://doi.org/10.1016/j.domaniend.2022.106771

Pan Y, Jing J, Qiao L et al (2018) MiRNA-seq reveals that miR-124-3p inhibits adipogenic differentiation of the stromal vascular fraction in sheep via targeting C/EBPalpha. Domest Anim Endocrinol 65:17–23. https://doi.org/10.1016/j.domaniend.2018.05.002

Pang T, Tang Q, Wei J et al (2022) Construction of a Novel Immune-Related prognostic-predicting model of gastric cancer. Gene 147032. https://doi.org/10.1016/j.gene.2022.147032

Park WY, Park J, Lee S et al (2022) PEX13 is required for thermogenesis of white adipose tissue in cold-exposed mice. Biochim Biophys Acta Mol Cell Biol Lipids 1867(1):159046. https://doi.org/10.1016/j.bbalip.2021.159046

Raghavan M, Del Cid N, Rizvi SM et al (2008) MHC class I assembly: out and about. Trends Immunol 29(9):436–443. https://doi.org/10.1016/j.it.2008.06.004

Rosen ED, Hsu CH, Wang X et al (2002) C/EBPalpha induces adipogenesis through PPARgamma: a unified pathway. Genes Dev 16(1):22–26. https://doi.org/10.1101/gad.948702

Roth D, Benz J, Grether U et al (2023) Homogeneous Time-Resolved Fluorescence Resonance Energy Transfer (TR-FRET) Cofactor Recruitment Assay for PPARalpha and PPARgamma. Methods Mol Biol 2576:155–169. https://doi.org/10.1007/978-1-0716-2728-0_12

Salazar-Onfray F, Charo J, Petersson M et al (1997) Down-regulation of the expression and function of the transporter associated with antigen processing in murine tumor cell lines expressing IL-10. J Immunol 159(7):3195–202

Sidhom EH, Kim C, Kost-Alimova M et al (2021) Targeting a Braf/Mapk pathway rescues podocyte lipid peroxidation in CoQ-deficiency kidney disease. J Clin Invest 131(5). https://doi.org/10.1172/JCI141380

Sun N, Liu D, Chen H et al (2012) Localization, expression change in PRRSV infection and association analysis of the porcine TAP1 gene. Int J Biol Sci 8(1):49–58. https://doi.org/10.7150/ijbs.8.49

Suzuki K, Yanagi M, Mori-Aoki A et al (2002) Transfection of single-stranded hepatitis A virus RNA activates MHC class I pathway. Clin Exp Immunol 127(2):234–242. https://doi.org/10.1046/j.1365-2249.2002.01767.x

Tanaka T, Yoshida N, Kishimoto T et al (1997) Defective adipocyte differentiation in mice lacking the C/EBPbeta and/or C/EBPdelta gene. EMBO J 16(24):7432–7443. https://doi.org/10.1093/emboj/16.24.7432

Tang S, Xie J, Wu W et al (2020) High ammonia exposure regulates lipid metabolism in the pig skeletal muscle via mTOR pathway. Sci Total Environ 740:139917. https://doi.org/10.1016/j.scitotenv.2020.139917

Vaske DA, Ruohonen-Lehto MK, Larson RG et al (1994) Rapid communication: restriction fragment length polymorphisms at the porcine transporter associated with antigen processing 1 (TAP1) locus. J Anim Sci 72(1):255. https://doi.org/10.2527/1994.721255x

Wang J, Hua L, Chen J et al (2017) Identification and characterization of long non-coding RNAs in subcutaneous adipose tissue from castrated and intact full-sib pair Huainan male pigs. BMC Genomics 18(1):542. https://doi.org/10.1186/s12864-017-3907-z

Wang Q, Song Z, Yang J et al (2022) Transcriptomic analysis of the innate immune signatures of a SARS-CoV-2 protein subunit vaccine ZF2001 and an mRNA vaccine RRV. Emerg Microbes Infect 11(1):1145–1153. https://doi.org/10.1080/22221751.2022.2059404

Xue P, Hou Y, Zuo Z et al (2020) Long isoforms of NRF1 negatively regulate adipogenesis via suppression of PPARgamma expression. Redox Biol 30:101414. https://doi.org/10.1016/j.redox.2019.101414

Zhang M, Shao Y, Gao B et al (2020) Erchen Decoction Mitigates Lipid Metabolism Disorder by the Regulation of PPARgamma and LPL Gene in a High-Fat Diet C57BL/6 Mice Model. Evid Based Complement Alternat Med 2020:9102475. https://doi.org/10.1155/2020/9102475

Zhang Y, Wu H, He R et al (2021) Dickkopf-2 knockdown protects against classic macrophage polarization and lipid loading by activation of Wnt/beta-catenin signaling. J Cardiol. https://doi.org/10.1016/j.jjcc.2021.04.010

Zhu K, Wang J, Zhu J et al (1999) p53 induces TAP1 and enhances the transport of MHC class I peptides. Oncogene 18(54):7740–7747. https://doi.org/10.1038/sj.onc.1203235

Zou C, Li S, Deng L et al (2017) Transcriptome Analysis Reveals Long Intergenic Noncoding RNAs Contributed to Growth and Meat Quality Differences between Yorkshire and Wannanhua Pig. Genes (Basel) 8(8). https://doi.org/10.3390/genes8080203

Zou C, Li L, Cheng X et al (2018) Identification and Functional Analysis of Long Intergenic Non-coding RNAs Underlying Intramuscular Fat Content in Pigs. Front Genet 9:102. https://doi.org/10.3389/fgene.2018.00102

Zubiria MG, Giordano AP, Gambaro SE et al (2020) Dexamethasone primes adipocyte precursor cells for differentiation by enhancing adipogenic competency. Life Sci 261:118363. https://doi.org/10.1016/j.lfs.2020.118363

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