Altered levels of histone deacetylases in HD mouse models

Zmiany poziomu deacetylazy histonów w mysich modelach choroby Huntingtona

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Wprowadzenie. Deacetylazy histonów (HDACs) w znacznym stopniu odpowiadają za wywoływane różnymi czynnikami patofizjologicznymi atrofie mięśni szkieletowych. Nadal nie jest jasne, czy w przebiegu atrofii mięśni związanej z chorobą Huntingtona (HD) występuje specyficzny profil transkryptów deacetylaz histonów.

Cel. Dokonanie walidacji poziomów transkryptów 11 deacetylaz histonów w mięśniu piszczelowym przednim (Tibialis anterior – TA) w dwóch mysich modelach HD.

Materiał i metody. Mięśnie TA pobrano z dwóch mysich modeli HD, celem wykonania łańcuchowej reakcji z użyciem odwrotnej transkryptazy służącej ilościowej ocenie transkryptu genu *Hdac*.

Wyniki. Wykryto istotne statystycznie podwyższone poziomy mRNA dla genów *Hdac1*, *Hdac2*, *Hdac4*, *Hdac5*, *Hdac6*, *Hdac8* i *Hdac11* w obu modelach mysich.

Wnioski. Wykryto zmiany w poziomie transkryptów HDACs, które są głównymi regulatorami epigenetycznymi. Czynniki te są nie tylko markerami molekularnymi patologicznej przebudowy mięśni szkieletowych, ale również molekularnymi celami dla nowych terapii, które mogą zredukować toksyczność huntingtyny.

Słowa kluczowe: *choroba Huntingtona, zanik mięśni szkieletowych, HDACs, biomarkery, modele mysie*

Introduction. Histone deacetylases (HDACs) play essential roles in the skeletal muscle atrophy that occurs in response to several pathophysiological stimuli. It remains unknown whether a common or unique transcriptional profile of HDAC genes exists during the progression of muscle atrophy in Huntington's disease (HD).

Aim. To validate the transcriptional level of 11 histone deacetylases in the Tibialis Anterior (TA) muscle of two HD mouse models.

Material & methods. Tibialis anterior muscles were harvested from two HD mouse models for a quantitative reverse transcriptase-polymerase chain reaction analysis of *Hdac* transcripts.

Results. We found mRNA levels of *Hdac1*, *Hdac2*, *Hdac4*, *Hdac5*, *Hdac6*, *Hdac8* and *Hdac11* to be significantly up-regulated in two fully symptomatic HD mouse models.

Conclusions. We identified transcriptional changes in HDACs that are major epigenetic regulators. These factors are therefore not only molecular markers of skeletal muscle pathological remodelling, but are potential targets for therapeutic intervention, to reduce HD toxicity.

Key words: *Huntington's disease, skeletal muscle atrophy, HDACs, biomarkers, mouse models*

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Introduction

Huntington's disease (HD) is a progressive neurodegenerative disorder for which there is no effective disease-modifying treatment [1]. As well as affecting the central nervous system, HD is a multi-system disease with a profound skeletal muscle malfunction [2, 3]. The disease is caused by the genetic expansion of a DNA CAG repeat to more than 35 CAGs, within exon1 of the *HTT* gene. This leads to a number of molecular events, including transcriptional dysregulation, which significantly contributes to disease progression [4]. In eukaryotes, it is known that global changes

in transcription are governed by a group of enzymes known as histone deacetylases (HDACs). Mammalian HDACs are a family of 18 proteins, divided into four groups based on structural and functional similarities: class I (HDACs: 1, 2, 3, 8), class IIa (HDACs: 4, 5, 7, 9), class IIb (HDACs: 6, 10), class III (sirtuins 1-7) and class IV (HDAC11 is the sole member); for a review, see [5]. In fact, alterations in the HDAC4 pathway have been described in the skeletal muscles of two HD mouse models (R6/2 and *Hdh*Q150) an have been found to be part of an important axis leading to HD muscle dysfunction [6]. R6/2 mice are transgenic for a mutated N-terminal exon 1 HTT fragment [7] while the *Hdh*Q150 mice have an expanded CAG repeat knocked-in to the mouse huntingtin gene (*Htt*) [8].

We are currently exploring the potential mechanisms by which HDACs might be involved in HD-related skeletal muscle atrophy. In this study we have investigated whether there are alterations in the transcriptional profiles of HDACs in the skeletal muscles of symptomatic 12 week-old R6/2 mice and 22-month old *Hdh*Q150 mice.

Aim

To validate the transcriptional level of 11 histone deacetylases in the Tibialis Anterior (TA) muscle of two HD mouse models.

Materials and methods

Mouse maintenance

The *Hdh*Q150 and R6/2 HD mouse lines were bred and genotyped as previously described. All experimental procedures were conducted under a project license from the Home Office, UK, and approved by the Animal Welfare and Ethical Review Body of Imperial College London. Experimental groups included the R6/2 and *Hdh*Q150 mouse models at 12 weeks and 22 months of age (N=6) and their littermates (N=6) respectively. All animals had unlimited access to water and breeding chow (Special Diet Services, Witham, UK), and housing conditions and environmental enrichment were as described previously [6].

RNA extraction and Taqman real-time PCR expression analysis

Total RNA from skeletal muscles (TA) was extracted with a mini-RNA kit (Qiagen), according to the manufacturer's instructions. The reverse transcription reaction (RT) was performed using MMLV superscript reverse transcriptase (Invitrogen) and random hexamers (Operon) [9]. All Taqman qPCR reactions were performed using the LightCycler® 480 Instrument (Roche). Estimation of mRNA copy number was determined in triplicate for each RNA sample by comparison to the geometric mean of three endogenous housekeeping genes, (Primer Design). Primer and probe sets for genes of interest were purchased from Primer Design or ABI. Primers for transcripts of *Hdacs* were previously described [10].

All Taqman qPCR values were normalized to the geometric mean of three housekeeping genes. Error bars are ±SEM (N=6). One-way ANOVA with Bonferroni post-hoc test: **p<0.01; ***p<0.001.

Statistical analysis

All data were analysed with Microsoft Office Excel and Student's t-test (two tailed) or one-way ANOVA SPSS (IBM).

Results

Typically, skeletal muscles respond to pathological processes or stresses by remodelling themselves in a manner that is associated with changes in epigenetic marks. This may be achieved by altering levels or activities of histone deacetylases (HDACs) [11]. We previously showed that the HDAC4 pathway is altered in HD skeletal muscles [6]. In the current studies we sought to profile the transcription of 11 *Hdacs* in two symptomatic HD mouse models with profound skeletal muscle atrophy. We performed transcriptional analysis of *Hdacs* on the cDNA library isolated from TA of 12 week-old R6/2 mice and 22 month-old *Hdh*Q150 mice.

We found a significant up-regulation of *Hdac1*, *Hdac2* and *Hdac8* mRNAs (class I members) in both HD mousse models. Among class IIa and b, we found a significant up-regulation of *Hdac4*, *Hdac5*, *Hdac6*, and while transcript levels of *Hdac7*, *Hdac9* and *Hdac10* remained un-changed. We constantly noticed a significant up-regulation of *Hdac6* mRNA (up to 3-fold) between both HD models. We also observed that *Hdac11* (the sole member of class IV) was significantly up-regulated in the TA HD muscles, in comparison to TA muscle from WT littermates (Figure 1 and 2).

Overall, this confirms that deregulated transcripts of *Hdacs* in HD skeletal muscles might contribute to the disease progression via epigenetic mechanisms related to changes in the transcriptional signature. However, this might be also mitigated by mechanisms linked to the non-histone acetylating actions of class II HDACs, including their well-described properties as transcriptional repressors; for a review see [12, 13].

Transcript levels of *Hdac1*, *Hdac2*, *Hdac4*, *Hdac5*, *Hdac6*, *Hdac8*, and *Hdac11* were increased, while *Hdac3*, *Hdac7*, *Hdac9* and *Hdac10* mRNAs remained unchanged.

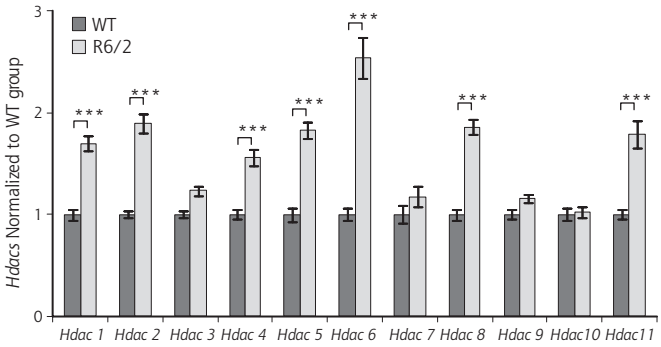

Fig. 1. Significant transcriptional deregulation of *Hdacs* in TA muscles of fully symptomatic R6/2 mice

Ryc. 1. Istotne zmiany w transkrypcji genów *Hdacs* w mięśniu TA objawowych myszy R6/2

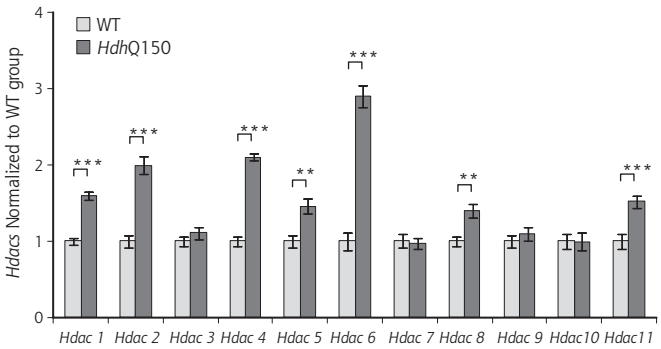

Fig. 2. Significant transcriptional deregulation of *Hdacs* in TA muscles of symptomatic *Hdh*Q150 mice

Ryc. 2. Istotne zmiany w transkrypcji genów *Hdacs* w mięśniu TA objawowych myszy *Hdh*Q150

Discussion

In the current studies we sought to establish whether there were any changes in the transcriptional signatures of *Hdacs* in atrophied skeletal muscles, using two well-established HD mouse models [6]. For the first time, we can report that the following *Hdacs* are significantly deregulated in the HD skeletal muscles: *Hdac1*, *Hdac2*, *Hdac4*, *Hdac5*, *Hdac6*, *Hdac8* and *Hdac11*. Interestingly, class I and in particular *Hdac1* has been already linked to skeletal muscle atrophy. It has been reported that HDAC1 is sufficient to activate FoxO transcription factors and induce muscle fiber atrophy *in vivo* [14]. Class IIa HDACs have also already been linked to skeletal muscle atrophy, likely through their repressive propensities via the MEF2 family of transcription factors [6, 12, 15]. HDAC6, a member of the class IIb HDACs that is significantly up-regulated in the TA of both HD mouse models has been recently identified as a valuable marker of muscle atrophy in mice and humans samples. Consequently, inactivation of HDAC6 in mice protects against muscle wasting [16].

Our data imply that HD-muscle malfunction might be governing the apparently different HDAC pathways and it would be worthwhile to elucidate involvement of particular *Hdacs* in disease progression. By understanding this complex pathological response better, it may perhaps be possible to intervene pharmacologically. Our study would suggest that lowering the activities of HDAC1, 2, 4, 5, 6, 8 and 11 might be beneficial in managing HD muscle atrophy. Hence, developing specific inhibitors that alter specific HDACs activities might be an improved therapeutic strategy.

Overall, in this study we aimed to verify whether there were any changes in epigenetic regulation in response to the intrinsic toxic effect of Htt mutant in skeletal muscles. The transcriptome showed evidence of dysregulation with striking changes in the HDAC genes. These transcriptional changes in major epigenetic regulators are not only molecular markers of skeletal muscle pathological remodelling, but are potential targets for therapeutic intervention, to reduce HD toxicity.

Conclusions

We identified transcriptional changes in HDACs that are major epigenetic regulators. These factors are therefore not only molecular markers of skeletal muscle pathological remodelling, but are potential targets for therapeutic intervention, to reduce HD toxicity.

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Piśmiennictwo / References

1. Zielonka D, Mielcarek M, Landwehrmeyer GB. Update on Huntington's disease: advances in care and emerging therapeutic options. Parkinsonism Relat Disord 2015, 21(3): 169-178.
2. Mielcarek M. Huntington's disease is a multi-system disorder. Rare Dis 2015, 3(1): e1058464.
3. Mielcarek M, Isalan M. A shared mechanism of muscle wasting in cancer and Huntington's disease. Clin Transl Med 2015, 4(1): 34.
4. Cha JH. Transcriptional signatures in Huntington's disease. Prog Neurobiol 2007, 83(4): 228-248.

5. Haberland M, Montgomery RL, Olson EN. The many roles of histone deacetylases in development and physiology: implications for disease and therapy. Nat Rev Genet 2009, 10(1): 32-42.
6. Mielcarek M, Toczek M, Smeets CJ, et al. HDAC4-myogenin axis as an important marker of HD-related skeletal muscle atrophy. PLoS Genet 2015, 11(3): e1005021.
7. Mangiarini L, Sathasivam K, Seller M, et al. Exon 1 of the HD gene with an expanded CAG repeat is sufficient to cause a progressive neurological phenotype in transgenic mice. Cell 1996, 87(3): 493-506.
8. Woodman B, Butler R, Landles C, et al. The Hdh(Q150/Q150) knock-in mouse model of HD and the R6/2 exon 1 model develop comparable and widespread molecular phenotypes. Brain Res Bull 2007, 72(2-3): 83-97.
9. Mielcarek M, Bondulich MK, Inuabasi L, et al. The Huntington's disease-related cardiomyopathy prevents a hypertrophic response in the R6/2 mouse model. PLoS One 2014, 9(9): e108961.
10. Mielcarek M, Benn CL, Franklin SA, et al. SAHA decreases HDAC 2 and 4 levels in vivo and improves molecular phenotypes in the R6/2 mouse model of Huntington's disease. PLoS One 2011, 6(11): e27746.
11. Colussi C, Illi B, Rosati J, et al. Histone deacetylase inhibitors: keeping momentum for neuromuscular and cardiovascular diseases treatment. Pharmacol Res 2010, 62(1): 3-10.
12. Mielcarek M, Zielonka D, Carnemolla A, et al. HDAC4 as a potential therapeutic target in neurodegenerative diseases: a summary of recent achievements. Front Cell Neurosci 2015, 9: 42.
13. Lehmann LH, Worst BC, Stanmore DA, Backs J. Histone deacetylase signaling in cardioprotection. Cell Mol Life Sci 2014, 71(9): 1673-1690.
14. Beharry AW, Sandesara PB, Roberts BM, et al. HDAC1 activates FoxO and is both sufficient and required for skeletal muscle atrophy. J Cell Sci 2014, 127(Pt 7): 1441-1453.
15. Moresi V, Williams AH, Meadows E, et al. Myogenin and class II HDACs control neurogenic muscle atrophy by inducing E3 ubiquitin ligases. Cell 2014, 143(1): 35-45.
16. Ratti F, Ramond F, Moncollin V, et al. Histone deacetylase 6 is a FoxO transcription factor-dependent effector in skeletal muscle atrophy. J Biol Chem 2015, 290(7): 4215-4224.