PROJECT REVIEW

"Molecular signatures of IGF-1 gene doping after AAV-mediated gene transfer"

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This is a highly interdisciplinary and coordinated project that is aimed at understanding the molecular modifications induced by prolonged IGF-1 gene expression in the skeletal muscle after viral mediated gene transfer in rodent animal models. The project will exploit the availability of AAV vectors expressing different IGF-1 isoforms to transduce skeletal muscles, a unique system that permits the long-term evaluation of the effects induced by the growth factor in vivo. In the treated animals, the signatures of these modifications will be analyzed by detecting the presence of the delivered transgenes in serum by quantitative real-time PCR and by analyzing the modifications of the proteomic pattern in muscle by advanced proteomics and mass spectrometry. These studies will be complemented by a parallel evaluation of the proteomic modifications induced by IGF-1 gene transfer in skeletal muscle satellite cells in vitro.

The project will be divided into three tasks.

Task 1. The overall purpose of this task will be the identification of a proteomic signature of IFG-1 gene transfer in muscle cells. The project will involve the development of animal models for AAV-mediated IGF-1 gene transfer. In particular, viral vectors expressing different IGF-1 isoforms will be produced and used to inoculate the tibialis anterior and femoral quadriceps muscles of mice and rats. At different times after transduction, proteomic analysis of the transduced muscles will be performed. These studies will take advantage of state-of-the-art technology 2-DE DIGE and will have the ultimate purpose of identifying protein patterns specific to the IGF-1-expressing muscles. These studies will be paralleled by the analysis of proteomic changes in human skeletal myoblasts after ex vivo gene transfer of the IGF-1 cDNAs.

Task 2. This task is aimed at the identification of novel peptide markers that might be exploited for anti-doping purposes. In particular, the project is aimed at the identification, quantification and qualitative assessment of the proteins differential expressed in muscles transduced with IGF-1. Differentially expressed or modified proteins will be analyzed using advanced mass spectrometry instrumentation, including MALDI-TOF/TOF, ESI-linear quadrupole ion trap and high resolution FT-MS instrumentation.

Task 3. This task is aimed at the assessment of gene doping by monitoring the presence of exogenous gene fragments in animal serum. This possibility is based on different experimental and clinical reports that indicate that muscle exercise determines significant damage to muscle cells, with the release of intracellular content into the bloodstream. At different times after in vivo IGF-I gene transfer, both in resting conditions and during muscle exercise, serum will be analyzed for the presence of promoter-, vector backbone- or cDNA-specific gene fragments by TaqMan-based, Real Time quantitative PCR.

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Results and Conclusions

In this project we set up a mouse model of gene doping by AAV-IGF1 injection into the mouse skeletal muscle. This model will be particularly useful for the identification of molecular signatures of gene doping, to be detected by a screening approach. IGF-1 overexpression at 1 month post-injection was found to induce extensive regeneration of the skeletal muscle, paralleled by a modest angiogenic response. We built a 2D-map of proteome of muscles overexpressing IGF-1 for different periods of time. Protein patterns analyzed by high resolution 2D-gel electrophoresis revealed that a vast number of proteins were influenced by AAV-IGF-1 transduction. The identification of these proteins was achieved using matrix-assisted laser desorption/ionisation-mass spectrometry, which indicated that the prolonged overexpression of IGF-1 induced marked modifications in the levels of several metabolic, structural, cytoskeletal, antioxidant and transport proteins, with a shift toward the oxidative metabolism along with diminished levels of enzymes involved in ROS production and with a significant increase in the levels of contractile proteins. We also developed a novel endurance test by using a swimming apparatus, and observed that mice injected in the limb muscles with AAV-IGF1 performed remarkably better than normal controls, consistent with the observation that IGF1 determined a switch of muscle fibers toward the slow type.

Finally, we also developed a cellular model for proteomic analysis in which IGF1 expression can be turned on pharmacologically in human muscle progenitor cells, engineered to conditionally express IGF1 using the TetOn system.

Overall, these results indicate that AAV-IGF-1 might be considered a powerful candidate for gene doping and warrant further investigation aimed at the detection of reliable markers for AAV-IGF-1 gene doping.