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THE VIRAL VECTOR WITH MUSCLE-SPECIFIC HYBRID PROMOTER AS A GENE THERAPY TOOL FOR LAMINOPATHIES

ABSTRACT

Muscular dystrophies belong to a heterogeneous group of incurable genetic illnesses affecting muscle tissue and the most promising potential treatment seems to be a gene therapy. In this thesis, the model system for chosen type – Emery-Dreifuss muscular dystrophy was developed, the strategy of therapy was proposed, the key elements of therapy were designed and the vector with genetic drug was prepared.

The genetic drug and model system. A fusion protein EGFP-prelamin A was chosen as an optimal drug at this experimental level. It is suitable to use with animal model system (*LMNA* knock-out mice) and cell culture model system. The model system was investigated in order to prepare conditions for future tests of therapy efficacy for dominant negative phenotypes. The cells from patient's biopsies were obtained. Human cell lines overexpressing lamin A mutants were prepared and investigated. Potential mechanisms preventing model system cells from high level overexpression were pointed out: specific lamin A removal from cells at early developmental stage and transient lamin C redistribution. The novel Emery-Dreifuss Muscular Dystrophy mutations L263P and D446V were characterized, including impact on phenotype development and proliferation rate.

The expression cassette. The novel promoter providing high level of expression in muscular cells was developed. The detailed analysis of particular functional elements was performed, the most important ones and the possible rearrangements in case of future need to increase specificity were demonstrated. Promoter's activity was analysed with different model systems: transient transfection with secretory luciferase or EGFP, lentiviral, AAV vector. Lack of toxicity of

cDNA encoding lamin A expression under control of novel promoter as well as its correct localization were demonstrated in muscle cells. The initial tests with animal model system were performed and confirmed high promoter's activity *in vivo*.

The carrier for genetic drug. The detailed analysis of available carriers was done basing on literature data, including clinical trials conclusions. Following viral carriers were tested: lentiviruses pseudotyped with different glycoprotein envelopes, adenoviruses and AAVs. As a carrier for cell culture tests and potential *ex vivo* therapy there was chosen the lentiviral vector pseudotyped with VSVg and its compatibility with expression cassette and transgen was examined. As a carrier for systemic and local therapy *in vivo* there was chosen AAV-9 and its compatibility with the novel expression cassette was also proven.

The vector with expression cassette was developed and it is possible to utilize it for gene therapy of different muscular dystrophies and other muscle-derived treatments, using various therapeutic strategies such as overexpression gene correction, silencing etc., including therapy for Emery-Dreifuss Muscular Dystrophy.