Histopathological characterization of skeletal muscles during the postnatal ontogenesis of dysferlin-deficient mice

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Gene therapy assays are fast-moving technology that requires well characterised animal model. There are many ways for gene transferring at the moment but none standardised protocol for efficiency and safety evaluation exists. The aim of our investigation is to distinguish histopathological features of mice strain with the absence of dysferlin expression.

Materials and methods. Gastrocnemius muscles of Bla/J (experimental group) and C57Bl/6 (control group) mice at the age of 1, 10, 20, 30 days, 2, 3, 4, 5, 7, 9, 12, 15, 18 months were obtained. Paraffin sections of calf muscles were stained with H&E, immunohistochemically with antibodies against myogenin (terminal myogenic differentiation). Lack of dysferlin expression was confirmed by PCR with primers to *dysf* gene and immunofluorescent staining with antibody to dysferlin protein.

Results. Necrotic muscle fibers (MF) were higher at all time points in experimental group from 2 to 18 months with gradual increasing of parameter (7,87±5,23% vs 15,6±4,78%, respectively, $p<0,005$), in C57BL/6 this parameter was significantly lower and did not exceed 1,2±1,33%. Percentage of centrinucleated MF in Bla/J was significantly higher from 5 (18,12±1,10% vs 0,13±0,42 in control, $p<0,05$) till 18 (25,56±10,69% vs 8,42±6,11% in control, $p<0,05$) months in Bla/J mice. Mean cross-sectional area was significantly higher in mutant mice from 1 to 7 months with gradual decreasing of parameter after 7 month. Meanwhile control group mean MF size increased after 9 month (1214,89±485,77% vs 712,39±467,45% in Bla/J, $p<0,05$). It was remarkable that percentage of myogenin positive nuclei in mutant mice increased till 4 month when in control group we observed inverse tendency.

Conclusion. Dysferlin-deficient animal model showed decreased regenerative potential of skeletal muscle with prolonged myogenic differentiation during natural history of disease. Obtained results would be useful for further gene therapy trials. Work supported by Program of Competitive Growth of KFU.