**Over-expression of BCL2 rescues muscle weakness in a mouse model of oculopharyngeal muscular dystrophy**

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**Objectives:**

The focus of this paper deals with preventing muscle damage due to oculopharyngeal muscular dystrophy (OPMD) by intentionally overexpressing the anti-apoptotic gene BCL2. A genetic condition, OPMD is a form of muscular dystrophy caused by a (GCG) codon expansion in the gene PABPN1, believed to give the resulting protein a toxic function. It is known, however, that mutated PABPN1 will cause the formation of tubule aggregates in skeletal muscle nuclei. The standard gene PABPN1 contains a stretch of 10 alanines in a row, with the first six being encoded by a (GCG) codon repeat. Cases of OPMD show an expansion of this repeat to anywhere between 8 and 13 codons, leading to an alanine stretch 12 to 17 units long. It is usually inherited in an autosomal dominant pattern. Symptoms coming from this disease include ptosis and dysphagia, developing proximal muscle weakness, and as the authors put it, “a severely impaired quality of life.”

Evidence collected suggests that OPMD might relate to cell apoptosis in mice models exhibiting OPMD; for instance, treatments of doxycycline, trehalose, and cystamine were shown in previous studies to both reduce muscle weakness and prevent apoptosis. Furthermore, transgenic mice expressing mutant PABPN1 showed apoptotic markers when analyzed. Still, apoptosis and its true connection to OPMD are uncertain.

Knowing that apoptosis and OPMD might be connected, the objective is to find out whether they are or are not truly connected and if prevention of apoptosis will reverse the damage that OPMD can cause. To do this, the gene B-cell CLL/lymphoma 2 (BCL2), an anti-apoptotic gene that antagonizes the activation of two pro-apoptotic proteins, BCL2-associated X protein (BAX) and BCL2 homologous antagonist/killer (BAK) to restrict apoptosis in mice expressing A17 OPMD.

**Experimental Approach and Results:**

To test if apoptosis and OPMD are negatively correlated, transgenic mice (A17) containing genes that encode for a 17-alanine stretch in PABPN1, the longest known expansion in cases of OPMD, were crossed with transgenic mice that over-express BCL2. The resulting F1 generation was composed of mice that were wild type, A17 expressing, BCL2 over-expressing, and A17 x BCL2. The mice were then genotyped and western blotting was performed to ensure the correct protein expression patterns for each type of mouse.

Over a period of 10 months, the mice were evaluated on several scales. Their grips (both forelimbs and all limbs) were tested with a grip strength meter. Characterizations such as maneuverability on a wire, vertical gripping, and pelvic elevation were examined by a series of behavioral tests referred to as SHIRPA. The collected results were then pooled and analyzed via statistical methods, such as the Mann-Whitney test, repeated-measure ANOVA, and the Chi-square test, for significance depending on which test was appropriate for each examination. Samples of muscle tissue were also taken from the mice and examined by methods in histology.

The results showed that the presence of over-expressed BCL2 helped to prevent muscle weakness in the first few months of life for A17 x BCL2 mice when compared to A17 mice, and that their grip and strength were greater until the end of the study in all examinations, between 9 and 10 months of age. These measures, however, did show poorer results than the wild type and only BCL2 mice, although their measures were improved over A17 mice. There was, however, a sharp decrease in the effectiveness of the crossbreeding as the test reached the ninth and tenth months. As the mice grew older, the A17 x BCL2 mice began to show results more similar to A17 mice than wild type mice.

A17 x BCL2 mice were also show to have larger weights over their entire lifespans. Their weights were, on average, between the lower weights of A17 mice and the higher weights of wild type and BCL2 mice. Samples of muscle mass showed that for both muscles reviewed: quadriceps and tibialis anterior, and at both 6 months and 11 months, the muscle masses of A17 x BCL2 mice were large enough to be statistically significant and greater than those of A17 mice at the same lifespan interval.

Histological results of the crossbred mice showed anti-apoptotic markers even later in life when their strength test results were similar to the results from A17 mice. The percentage of TUNEL-positive nuclei, nuclei that show pro-apoptotic characteristics, within the muscles of the mice were much higher in A17 mice than in A17 x BCL2 mice at both 6 months and at 11 months. Unusual to these results, however, was the fact that tubular filament aggregates in the nucleus were more present in A17 x BCL2 mice than A17 mice, when this is a known effect of OPMD. It is believed, however, that this result is due to the cell life increase conferred by BCL2, allowing OPMD cells to live longer by not entering apoptosis. The presence of diffuse cytochrome c, normally found in the mitochondria—which is dissolved by BAK and BAX during apoptosis—was also used as an indicator for apoptosis, and showed to be much higher in A17 mice than A17 x BCL2 mice and wild-type mice. The latter two showed no significant difference to each other, but a significant difference from A17. This result was the same in a test for caspase 3, a complex associated with apoptosis. However, in observing the results for scoring centralized nuclei, another test for apoptosis in muscular tissue, A17 and A17 x BCL2 mice showed no significant difference, and were significantly different from wild type mice.

**Conclusion:**

It can be stated that over-expression of BCL2 did rescue muscle weakness in the mice. Prevention of apoptosis, confirmed by the many tests that showed A17 mice had a much higher rate of apoptosis than A17 x BCL2 mice, proved to prevent muscle loss because of the disease. However, a catch was also revealed by this: apoptosis is not the sole cause of cell death in OPMD. The weakening of A17 x BCL2 mice in later stages illustrated that more than apoptosis must be at the root of OPMD’s symptoms.

Out of this research, the authors developed a new mechanism that illustrates the roles of PABPN1, BCL2, and mutant PABPN1 in muscle cell apoptosis. In this mechanism, the mutant PABPN1 activates BAX, which is inactivated by BCL2. BAX then breaks down mitochondria walls and releases cytochrome c, which triggers a series of reactions leading to caspase 3 and eventually apoptosis. PABPN1 is thought by the authors to inhibit caspase 3 by activating a complex called X-linked inhibitor of apoptosis (XIAP).

**Future Research Needed:**

There are a few critiques to be made about the research presented. Most notably, the paper mentions the use of anti-apoptotic drugs being used in the treatment of OPMD, as they were shown to rescue muscle strength and also decrease apoptosis at the same time. The authors then lend more argument to this point with the study, and emphasize it in their conclusion, yet discourage their own method of experimentation with BCL2 to antagonize apoptosis due to difficulty in utilizing the products in living beings and making them transient. If this were the case, the point for using transgenic mice expressing BCL2 seems to have eroded away in favor of a more realistic scenario of examining mice cells and strength of A17 mice who are being treated with the drugs that were already known to have these effects. Another criticism worthy of note was the use of solely A17 mice, and no A12 mice, A13 mice, etc. Though a polyalanine repeat of 12 to 17 alanines in PABPN1 is known to cause OPMD, that is not to say there is not a possibility that the way OPMD and BCL2 react together do not differ among the number of alanines conferred in the mutant protein. There is also a little bit of criticism to be made on the SHIRPA behavioral tests, as these tests are categorized and thus the differences between each category is arbitrarily set as opposed to a presence-absence test. Being that the results likely are also dependent on the behavioral patterns of the mice, results from these tests may be an approximate of actual values, at best.

Still, this research opens up quite a few doors of possibility. With apoptosis linked to OPMD, yet it being illustrated as not the only factor in cell death due to the disease, what other factors are there? Discovering these facts and their treatment or prevention could revolutionize treatment of OPMD in humans. Likewise, anti-apoptotics could be trialed and eventually marketed as a treatment for OPMD, provided medical side effects do not turn out to be too great. The authors also theorized that apoptosis may not be unique to OPMD in terms of muscular dystrophy, which means that, with future research, other forms of muscular dystrophy could be better understood due to what research was done here and perhaps someday, with all of this knowledge, muscular dystrophy will be a disease of the past.