essential for proper cardiac function. Mechanical and electrical activities must synchronize so that the work of individual cardiomyocytes transforms into the pumping function of the heart. Gap junctions are specialized cell-cell junctions that mediate inter-cellular communication. They are composed of connexin proteins, which form transmembrane channels for small molecules. Here, we showed that altered distribution of connexin 43 occurs prior to any electrical disturbances in a mouse model of dilated cardiomyopathy due to LMNA mutations. We next assessed in vitro the molecular mechanisms of connexin 43 re-localization in pathological context. We showed that the presence of LMNA mutations triggers an abnormal trafficking of connexin 43 along both microtubules and actin networks leading to a loss of cell-cell communication. Going further, we demonstrated that modulating this process could restore the correct localization and function of connexin 43 at the cell-cell junction in cardiomyocytes carrying LMNA mutation. Our work could break new ground for future work towards developing novel treatment for malignant arrhythmias.

LMNA, A-type lamins, arrhythmias, dilated cardiomyopathy, connexin 43

Nuclear envelopathies (lamin A/C, emerin, others)- #2845

P21- 329- Defects in actin polymerization participate in cardiac contractility in Emery-Dreifuss muscular dystrophy

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Mutations in the lamin A/C gene (LMNA), encoding nuclear envelope proteins, cause autosomal dominant Emery-Dreifuss muscular dystrophy (EDMD), by mechanisms that remain incompletely understood. We previously discovered abnormally stress-activated elevated extracellular signal-regulated kinase 1/2 (ERK1/2) activities in heart in EDMD. This is a cornerstone in the development of heart disease in this muscular dystrophy. However, the understanding of molecular and cellular mechanisms underlying the modulation of ERK1/2 signaling in the heart caused by LMNA mutation remains totally unclear. We here showed that there is an aberrant cytoplasmic localization of active (phosphorylated) form or ERK1/2 in cellular and animal models of EDMD. We next identified a novel interaction between cytoplasmic p-ERK1/2 and cofilin-1, an actin-depolymerizing protein, which in turn phosphorylates and activates cofilin-1 on a previously un-described phosphorylation site. This event triggers alteration of actin dynamics in both cellular and murin models of EDMD. These events could be blunted with the overexpression of a mutated cofilin-1 (phospho dead mutant). These findings unravel a novel role played by ERK1/2 signaling in actin dynamics that provide a novel insight into the disease etiology for the cardiac phenotype in EDMD and lay the groundwork for new therapeutic strategies.

LMNA, A-type lamins, dilated cardiomyopathy, actin, cofilin

Nuclear envelopathies (lamin A/C, emerin, others)- #3038

P21- 330- FHL1B, a protein involved in Emery-Dreifuss muscular dystrophy, is a nuclear envelope protein.

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Mutations in the EMD and LMNA genes encoding the two nuclear envelope (NE) proteins emerin and lamin A/C are associated with the hereditary muscular disease Emery-Dreifuss muscular dystrophy (EDMD). Our group described the first mutations in the Four-and-a-Half LIM domain 1 (FHL1) gene to be responsible for EDMD. FHL1A and its spliced variants FHL1B and FHL1C have been so far reported to belong to the non-nuclear envelope proteins. To try to better understand the role of FHL1 protein isoforms in humans, we studied the precise expression and localization of the FHL1 isoforms during primary human myoblast differentiation.
We showed that in human control myoblasts, from the three FHL1 isoforms, only FHL1B displayed a clear accumulation at the inner nuclear membrane (INM), co-localized with lamin A/C and emerin. Differentiation of myoblasts into mature myotubes caused a progressive decrease of FHL1B from the nucleus which is not due to its nuclear export, but is rather due to a drop in its expression, probably reflecting the temporal role of FHL1B in myoblast differentiation. Interestingly, we found the expression of FHL1B to be affected in myoblasts of a patient with FHL1-associated EDMD that we previously reported to have differentiation defects.
FHL1B localization at the INM is not affected in myoblasts of patients carrying mutations in NE proteins (emerin, lamin A/C and nesprin-1) and in human myoblasts with lamin A/C knock-down, leading to the conclusion that FHL1B localization at the INM is independent of emerin or lamin A/C. However, we found an upregulation of FHL1B expression in a patient carrying a heterozygous mutation in LMNA.
Collectively, we showed for the first time that FHL1B is an INM protein in myoblasts, like emerin and lamin A/C, the two other proteins involved in EDMD, and that its expression level probably needs to be decreased for myoblast differentiation to occur. Altogether, we recommend that future studies in the attempt to understand the underlying mechanism of FHL1-related EDMD should target each FHL1 protein isoform separately.

FHL1, FHL1B, Lamin A/C, Emerin, EDMD, Nuclear Envelope

P22 – Pharmacological therapies- N° 331 to N° 337

Pharmacological therapy of neuromuscular disease- #2450

P22- 331- Effects of Fish-oil supplementation on glucocorticoid receptor phosphorylation during dexamethasone-induced muscle atrophy.

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Many conditions are related with muscle atrophy, such as inactivity, sepsis, diabetes, cancer and corticosteroid therapy, leading to an increase of protein degradation and/or reduction of protein synthesis involving at least five systems: lisossomal, ubiquitin-proteasome, calpains, caspases and metalloproteinase. Glucocorticoid is one of the most prescribed drugs and its long-term use is related to muscle atrophy. Many studies have been searching for supplements in order to prevent this side effect; however, previous study has shown that fish-oil can aggravate the dexamethasone-induced muscle atrophy. Considering this, the goal of this study was to assess whether fish-oil supplementation (EPA + DHA) would affect the glucocorticoid receptor (GR) phosphorylation (Ser211), correspondent with GR activity during dexamethasone-induced muscle atrophy. Methods: Treated and non-treated rats with fish-oil (40 days) were subjected to dexamethasone administration, forming 4 groups: CT (control); DX 2,5 (dexa 2,5); FO (fish-oil) and DX+FO (dexa 2,5 + fish-oil). Muscles were extracted to cross sectional areas evaluation and P-GR (ser211) western blot analysis. Dexa administration led to a reduction around 47% on cross sectional area of 2B muscle fibers type (DX 2,5 and DX+F-O groups). DX+FO group showed a significant muscle atrophy on 1 an 2A fiber types in comparison to the other groups, including DX 2,5 group. P-GR (Ser211) evaluation showed a higher relative expression on DX 2,5 + FO group compared to other groups, corresponding to the observed labeling of P-GR by immunohistochemistry on tibialis anterior, with stronger nuclear labeling on DX+FO group in comparison to the others. Conclusions: Previous and concomitant administration of FO with DX 2,5 caused aggravation of muscle atrophy (mainly in 1 and 2A fiber types) associated to increased glucocorticoid receptor activity on Ser211, however, further tests would be useful to better understand the aggravation related to fish-oil supplementation. While the fish-oil is known to be effective in attenuating the muscle atrophy induced by sepsis and cancer, its concomitance with glucocorticoid can aggravate its side effects to skeletal muscle, probably GR-mediated. The identification of nutritional supplements able to alleviate the side effects of corticosteroids on skeletal muscle and the potential molecular pathways involved in this process, would be very important in the medical practice. FAPESP: 2013/23191-6

Skeletal muscle; glucocorticoid; muscle atrophy; fish oil; omega-3.

Pharmacological therapy of neuromuscular disease- #2459

P22- 332- Repurposed cancer therapeutics as treatments for DMD

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By studying the fate of the dystrophin glycoprotein complex in Duchenne muscular dystrophy (DMD) we have identified tyrosine phosphorylation of dystroglycan, the key transmembrane laminin receptor, as central to the loss of the entire DGC from the sarcolemma.
Preventing phosphorylation of dystroglycan in mdx miceby mutationof a key tyrosine phosphorylation site ameliorates the dystrophic phenotype. Studies in mouse myoblasts also demonstrate that pharmacological treatment with proteasome or tyrosine kinase inhibitors can increase levels of non-phosphorylated dystroglycan. Furthermore by inhibiting tyrosine phosphorylation, ubiquitination or proteasomal degradation pharmacologically we can demonstrate a reduction in dystroglycan phosphorylation and a rescue of the dystrophic phenotype in sapje zebrafish, a fish model of DMD. Through the use of FDA approved cancer therapeutics, we can demonstrate significant improvement in sapje zebrafish swimming ability when treated with either tyrosine kinase or proteasome inhibitors.
We have extended these studies into mdx mice and again certain drug regimen demonstrate improvements in muscle pathophysiology including muscle central nucleation, serum creatine kinase levels, restoration of dystroglycan and sarcoglycan to the sarcolemma and in physical parameters such as wire hanging times.
These studies demonstrate the utility of inhibiting dystroglycan tyrosine phosphorylation as a therapeutic strategy for DMD, particularly as several of the compounds that are effective are in existing clinical use. Obtaining orphan drug status for repurposed drugs could be a rapid and effective route to DMD therapy, either in their own right or as adjuncts to other therapies currently in or nearing the clinic.

Dystroglycan, Phosphorylation, Src, Tyrosine kinase inhibitors, myoblasts, zebrafish, mdx mouse

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Pharmacological therapy of neuromuscular disease- #2642

P22- 333- Treatment effect of idebenone on inspiratory muscle function in patients with Duchenne muscular dystrophy

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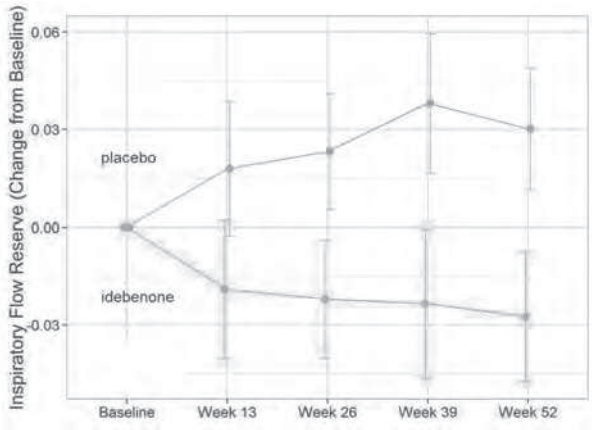
13. Array, Array, Array,

Background. Serial measurement of lung function is an important part of the standard of care of patients with Duchenne muscular dystrophy (DMD). In DMD patients, lung function is generally assessed by measuring lung volumes (e.g. forced vital capacity [FVC] and forced expiratory volume in one second [FEV1]) and peak expiratory flow [PEF]. However, direct assessment of dynamic inspiratory muscle function can provide additional information about the degree and progression of pulmonary involvement in this patient population. In a Phase 3 placebo-controlled clinical trial (DELOS), idebenone reduced the

loss of lung function (as assessed by PEF, FVC and FEV1) in 10-18 year old DMD patients not using concomitant glucocorticoid steroids (Lancet 2015;385:1748-57). Here we evaluate the effect of idebenone on parameters of inspiratory muscle function. Methods. We evaluated the effect of idebenone on the highest flow generated during an inspiratory FVC maneuver (maximum inspiratory flow; V'I,max(FVC)) and the ratio between the largest inspiratory flow during tidal breathing (tidal inspiratory flow; V'I,max(t)) and the V'I,max(FVC)- also termed Inspiratory Flow Reserve (IFR). Findings. DMD patients in both treatment groups of DELOS (idebenone, N=31; placebo: N=33) had comparable and abnormal V'I,max(FVC) at baseline. During the one-year study period, V'I, max(FVC) declined in patients on placebo, with a magnitude of change from baseline that approached statistical significance at week 26 (p=0.057) and reached statistical significance at weeks 39 (p=0.007) and week 52 (p=0.008). Conversely, in patients in the idebenone group the V'I,max(FVC) remained stable throughout the study period (p=0.95 at week 52). The between-group difference in favor of idebenone was 0.27 L/s (p=0.04) at week 26 and 0.30 L/s (p=0.06) at week 52. During the one-year study period, the IFR improved by 2.8% in patients receiving idebenone and worsened by 3.0% among patients on placebo with a between group difference of 5.8%, which was statistically significant at week 52 (p=0.04). Interpretation. Inspiratory muscle function is abnormal in 10-18 year old patients with DMD not taking glucocorticoid steroids. Over the 1-year study period of DELOS, idebenone reduced the loss of pulmonary function as assessed by V'I,max(FVC) and IFR, suggesting a protective effect on the diaphragm. These findings corroborate the efficacy of idebenone in preserving respiratory muscle function in patients with DMD.



Duchenne, respiratory disease, idebenone, randomised controlled trial, treatment

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Pharmacological therapy of neuromuscular disease- #2675

P22- 334- Idebenone reduces respiratory complications and antibiotic use in patients with Duchenne muscular dystrophy

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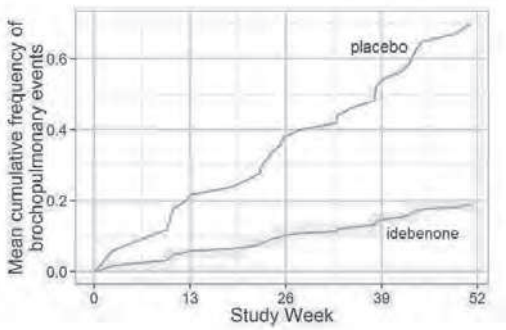
Background. In DMD, progressive weakness of respiratory muscles leads to restrictive pulmonary disease that evolves into severe respiratory complications. Of particular concern are ineffective cough, secretion retention and recurrent respiratory tract infections, which contribute to morbidity and mortality. In a Phase 3 clinical trial (DELOS), idebenone, a short-chain benzoquinone, reduced the loss of pulmonary function in DMD patients 10-18 years of age not taking concomitant glucocorticoid steroids over a 52-week period (Lancet 2015;385:1748-57). Methods. In a post-hoc analysis of DELOS, ?bronchopulmonary adverse events? (BAEs) were defined by a study-independent physician blinded to treatment assignment. BAEs included bronchitis, pneumonia, upper respiratory tract infection, influenza and/or viral infection with respiratory symptoms, laryngitis, respiratory failure, cough and dyspnea. The proportion of patients affected was compared between the idebenone (N=31) and placebo (N=33) groups. In addition, use of antibiotics for the treatment of BAEs was also assessed. Findings. More patients in the placebo group than in the idebenone group reported BAEs (17 of 33 patients, 27 events vs. 6 of 31 patients, 7 events) during the 52-week treatment period. The Hazard ratios calculated ?by patient? (HR 0.33; 95%CI: 0.13-0.84, p=0.02) and for ?all AEs? (HR 0.27; 95%CI: 0.12-0.62, p=0.002) indicated a clear idebenone treatment effect. The overall duration of such BAEs was 211 days in the placebo group vs. 82 days in the idebenone group. In addition, there was also a difference in the use of systemic antibiotics. In the placebo group 13 patients (39.4%) reported 17 episodes of antibiotic use compared to 7 patients (22.6%) reporting 8 episodes of antibiotic use in the idebenone group (HR for patients: 0.52; 95%CI: 0.21-1.30, p=0.16; HR for episodes: 0.53; 95%CI: 0.23-1.22, p=0.13). Furthermore, patients in the placebo group used systemic antibiotics for longer (105 days) compared to patients in the idebenone group (65 days). Interpretation. This post-hoc analysis of DELOS indicates that idebenone reduces the risk of bronchopulmonary adverse events. In addition, idebenone treatment was associated with reduced need for systemic antibiotics. These finding are clinically relevant as respiratory complications are a major cause of morbidity and mortality in DMD, and underscore the clinical meaningfulness of the idebenone treatment effect on respiratory function parameters.



Duchenne, idebenone, respiratory, randomised controlled trial

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Pharmacological therapy of neuromuscular disease- #2913

P22- 335- Diapocynin, a putative NADPH oxidase inhibitor, ameliorates the phenotype of a mouse model of Duchenne muscular dystrophy

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Duchenne muscular dystrophy (DMD) is a severe X-linked muscular disease that causes premature death and for which no cure exists. We have shown previously that in vitro treatment of dystrophic myotubes and excised muscles with diapocynin, a dimer of the classically used NADPH oxidase inhibitor apocynin, ameliorated several molecular events involved in DMD pathogenesis, of which ROS production, phospholipase A2 activity, Ca2+ influx and sarcolemmal integrity.
Here, we report on the in vivo effects of diapocynin and apocynin in mdx5Cv dystrophic mice, a model of DMD. Apocynin (50 mg/kg/day) and diapocynin (10 and 100 mg/kg/day) were given orally to mdx5Cv mouse pups, first via the lactating mothers from post-natal day 14 to 28 and subsequently directly to the weaned pups till post-natal day 35±1 or 60±3. Diapocynin but not apocynin enhanced spontaneous locomotor activity, rescued voluntary wheel running capabilities, and ameliorated diaphragm structure of dystrophic mice. Diapocynin and apocynin were equally potent at increasing the resistance to fatigue of triceps surae muscles exposed to repeated isometric contractions in situ and at preserving sarcolemmal integrity as evidenced by Evans blue dye uptake. Furthermore, microarray analyses showed a marked trend of the treatments to correct gene expression in dystrophic mice towards wild type controls.
Although apocynin and diapocynin had beneficial effects in dystrophic mice, diapocynin was superior in improving locomotion. Our findings suggest that diapocynin holds therapeutic potential for DMD.

NADPH oxidase, diapocynin, duchenne muscular dystrophy, reactive oxygen species,mdx

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Pharmacological therapy of neuromuscular disease- #2915

P22- 336- Rimeporide restores resting pH and decreases store operated calcium entry in dystrophic myotubes by inhibiting sodium hydrogen exchanger type 1 (NHE 1)

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Rimeporide is a specific Na+/H+ exchanger type 1 (NHE?1) inhibitor, which was originally developed up to Phase I as a treatment for congestive heart failure. There is considerable overlap in the pathophysiological changes observed in CHF and those observed in Duchenne muscular dystrophy (DMD). Thus, NHE?1 inhibition represents a promising and novel therapeutic approach to alleviate disease progression in patients with DMD as suggested by a study using another NHE inhibitor (Iwata et al, 2007).
In this project we first aimed to quantify the expression level of NHE?1 in primary cultures of dystrophic mice myotubes and to study the ?on target? and ?off-target? effects of rimeporide. Using western-blots, we confirmed an over-expression of NHE?1, the molecular target of rimeporide, in primary cultures of myotubes from mdx5Cv dystrophic mice, a murine model of DMD. The ?on target? effects of rimeporide on intracellular pH and Na+ fluxes were evaluated, both in basal conditions and after an NH4Cl pre-pulse, a procedure used to exacerbate NHE activity. We found that the cytosol of dystrophic myotubes was slightly more alkaline than that of wild type myotubes. This finding, together with distinct dynamic responses of the myotubes to NH4Cl stimulation strongly suggests that NHE?1 activity is enhanced in dystrophic muscle cells and that NHE-1 overexpresion contributes to the increased pH at rest. This correlates with the impaired recovery from acidosis observed in patients with DMD (Torriani et al, 2012). In addition, we found that rimeporide-mediated inhibition of NHE?1 caused correction of the resting pH levels. We finally explored ?off-target? effects of NHE-1 blockade by rimeporide in particular on the ability of the myotubes to handle Ca2+ and to generate reactive oxygen species. Rimeporide triggered a moderate inhibition of Ca2+ entry in myotubes through store-operated calcium channels and to a lower extent through stretch activated channels, which together with the correction of pH and regulation of intracellular sodium, may result in a therapeutic benefit in DMD patients. We are conducting an in vivo study in mdx mice to further explore its therpeutic potential.
The present results combined with those from in vivo efficacy studies in mdx mice and cardiomyopathic hamsters, led the European Medicines Agency to grant an orphan drug designation to rimeporide in DMD. Rimeporide safety and tolerability is currently evaluated in a phase Ib study in patients with DMD.

rimeporide, sodium hydrogen exchanger, pH, duchenne, pharmacotherapy

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Pharmacological therapy of neuromuscular disease- #2924

P22- 337- Preclinical evaluation of tamoxifen and other selective estrogen receptor modulators in mdx5Cv dystrophic mice.

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We are investigating the effects of selective estrogen receptor modulators (SERMs) in mdx5Cv dystrophic mice (Dys), a model for Duchenne muscular dystrophy (DMD). SERMs display either pro-estrogenic or anti-estrogenic activities in a tissue-dependent manner. Tamoxifen (TAM), the most well characterised SERM, has been used for over 30 years to treat estrogen-sensitive breast cancer in both women and men and has been reported to be also well tolerated in pre-pubertal boys.
In 2013, we published that oral treatment of Dys mice from 3 weeks of age for 15 months with TAM (10 mg/kg/day) improved muscle force and the structure of diaphragm and heart. TAM and its metabolites were present in nanomolar concentrations in plasma and muscles, suggesting signalling through high affinity targets, likely the estrogen receptors alpha and beta that were several-fold more abundant in dystrophic muscle than in normal ones.
Next, we tested TAM in adult Dys mice in order to investigate its efficacy in the low-intensity chronic stage of the disease, which resembles most closely the DMD condition. TAM at doses as low as 0.1 mg/kg/day improved motor performance of active mice and enhanced the contractile characteristics of the triceps surae. At 3 mg/kg/day, TAM corrected most endpoints close to normal values.
We are currently testing other SERMs (all at 3 mg/kg/day): the chlorinated TAM analogues clomiphene and toremifene, the 3-hydroxylated TAM derivative droloxifene, the second generation SERM unrelated to TAM raloxifene (RAL), and the pure anti-estrogen fulvestrant (Faslodex). Overall, the ranked efficacy was as follows: TAM > toremifene > clomiphene > droloxifene ? RAL > Faslodex.
Our data as well as our current understanding of estrogenic signalling in dystrophic muscle suggests that TAM and other SERMs with pro-estrogenic activities on muscle might be beneficial for DMD and maybe also for other muscular dystrophies.

Dystrophic mouse, pharmacotherapy, tamoxifen, SERM, force

P23 – Skeletal muscle development- N° 338 to N° 358

Skeletal muscle development- #2414

P23- 338- An efficient RNA interference screening, using C2C12 line, identifies new genes involved in myogenic differentiation.

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Skeletal muscle is a complex and heterogeneous tissue serving a multitude of functions in the organism. This tissue forms by a highly ordered process: myogenesis, which can be subdivided into a sequence of temporally separable events: myoblast proliferation, cell fusion and myotube maturation into myofibers. Although myogenesis has been widely described, many genes involved in muscle cell proliferation/differentiation are still unknown. To identify novel genes involved in this process, we have developed a functional screening system, based on the use of RNAi technology and C2C12 line (myoblast cell line, which is a very useful tool to study aspects of myogenesis, metabolism and muscle biology). This systematic genetic approach consists in the identification of genes that when knocked-down by RNAi show various phenotypes during proliferation and/or differentiation of C2C12. The knockdown of genes involved in C2C12 proliferation and/or differentiation might modify nuclear number and/or myosin quantity, which were easily detected by immuno-fluorescence staining (myosin antibody) and DAPI. The quantification of such staining indicates if the inactivated gene enhances or blocks proliferation, differentiation or both. In our screen, 100 mouse genes with an unknown function were knocked-down in C2C12 cells. The observed phenotypes were classified according to the following criteria: (i) nucleus counting which reflect the proliferation stage; (ii) myosin quantification, myotube morphology and fusion index which reflect the different steps of the differentiation stage. The screening result shows that among the 100 genes knocked-down, 92 genes display a phenotype. The phenotypic analysis indicates that 4 genes are specifically involved in proliferation stage, 45 genes are essential to the differentiation stage and 43 genes seem to be necessary as well for the proliferation as for the differentiation stages. Our results indicate that RNAi screening appears to be an efficient tool to identify new genes having a role during myogenesis.

Muscle, myogenesis, RNAi, screening, C2C12

Skeletal muscle development- #2431

P23- 339- Distinct branches of Wnt signaling control Neuromuscular Junction formation

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Understanding the developmental steps shaping the formation of the specialized peripheral synapse connecting motoneurons to skeletal muscle fibers, also called neuromuscular junction (NMJ) is critical. Recently, growing evidences suggest that Wnt morphogens act as key players in the formation of this synapse. Yet, the collaborative function of specific Wnts and downstream signaling at the NMJ remain poorly understood. We demonstrate that Wnt11 is required for the early nerve-independent muscle prepatterning, a process characterized by acetylcholine receptors (AChR) clustering in discrete domains of the muscle surface. Moreover, both Wnt4 and Wnt11 cooperate to enhance AChR clustering in muscle cell line and in diaphragm in vivo, in part via activation of the canonical pathway. In addition, in utero injections of specific secreted Wnt signaling inhibitors lead to similar pre