THE SKELETAL MUSCLE

The skeletal muscle is composed of striated muscle tissue under voluntary control and plays a key role in many activities such as the maintenance of posture, the locomotion, the speech and the breathing. The heart is a striated muscle but cardiac cells, of which it is composed, are under involuntary control and modulated by the autonomic nervous system.

The skeletal muscle tissue is about 40% of the total weight of an adult and is formed by contractile and multinucleated muscle cells, called muscle fibers or **myofibers**, formed by the fusion of **myoblasts**. Each myofiber is surrounded by the endomysium or **basal lamina**; the bundles of myofibers are surrounded by the **perimysium**, while the whole muscle is contained within the **epimysium**. Each myofiber is then attached at its ends to the tendons thanks to the myotendinous junctions. Myofibers are composed of myofibrils of **myosin** and **actin** repeated to form the **sarcomere**, the basic functional unit of the skeletal muscle.

In the neonatal stage, the number of muscle fibers remains constant but each myofiber grows in size thanks to the fusion of muscle stem cells, called **satellite cells**.

THE PROCESS OF MUSCLE REGENERATION: KEY ROLE OF SATELLITE CELLS

The adult mammalian skeletal muscle demonstrates an extraordinary ability to adapt to physiological changes, such as growth, physical exercise and tissue damage. The processes by which these changes are possible, are widely attributed to a small population of cells located in skeletal muscle and named **satellite cells**.

This kind of cells are called satellite since they are localized between the basal lamina and the plasma membrane of the myofiber, called sarcolemma, and are morphologically characterized by a high ratio of nucleus/cytoplasm, small nucleus and few organelles. In health, muscle satellite cells are in a quiescent non-proliferative state. Quiescent satellite cells express specific factors, useful as markers to identify and characterize them and for purification of the cell population. Among these, the most important is the transcription factor **Pax7**, which is essential for the survival of satellite cells.

In response to different stimuli, such as trauma, muscle satellite cells are activated, they begin to proliferate and express specific myogenic markers. The progression of satellite cells to activate the myogenic differentiation is mainly controlled by specific factors: **Myf5** and

MyoD and it ends with the expression of **myogenin** that drives the fusion of these cells **i**) to the existing muscle fibers **ii**) between them to form new myofibers with the final aim of regenerating the damaged skeletal muscle. The factors Myf5, MyoD and myogenin include the so-called group of myogenic regulatory factors, since they are the main genes responsible for the commitment of satellite cells for muscle regeneration.

During the muscle regeneration process, satellite cells may undergo different destinations, finely governed by gene expressions over time of the appropriate factors: a part of the cells is induced to differentiate (80%), the other one returns to a quiescent state to maintain the progenitor pool (20%).

MUSCLE DISEASES

The scientific community is making many efforts to better understand the cellular and molecular mechanisms that occur during muscle regeneration because such knowledge could help develop new therapies for diseases characterized by degeneration of skeletal muscle. Genetically determined muscular diseases such as muscular dystrophy, or regressive, such as sarcopenia, greatly affect the quality of life and are associated with increased morbidity and mortality.

1) MUSCULAR DYSTROPHY

Muscular dystrophy is characterized by progressive muscle degeneration and subsequent weakening of voluntary muscles which leads to the restriction of mobility of the subject, leading to confinement in a wheelchair and, in severe forms, to cardiac and respiratory failure. Muscular dystrophy is a group of inherited diseases that, in general, are caused by mutation of a gene encoding proteins of the cytoskeleton or membrane.

The most common and severe of these diseases is Duchenne muscular dystrophy (DMD), which is caused by mutation of the gene encoding **dystrophin**, a fundamental part of the complex that binds the intracellular cytoskeleton with the extracellular matrix in the muscle. This disease affects children in the early years of life, bringing total immobility within thirty years and causing considerable difficulties in respiratory and cardiac function .

Muscular dystrophy is one of the most difficult diseases to treat because of the skeletal muscle is formed by long multinucleated fibers whose nuclei can’t divide; hereafter, the

scientific community is trying to direct research towards a cellular therapy to restore the correct expression of the mutated gene .

2) SARCOPENIA

Sarcopenia is a syndrome characterized by progressive and generalized loss of muscle mass and strength, with the risk of serious negative consequences such as physical disability, worsening of quality of life and death.

The loss of muscle mass is a natural age-related factor, often caused by an unbalanced diet, a reduction in protein synthesis, physical inactivity, by slowing basal metabolic rate and the infiltration of fat in the muscle tissue.

Sarcopenia begins to appear at around 40 years old with the progressive reduction of the number and the size of muscle fibers, and with the increase of the fat and connective component. This syndrome affects about 30% of adults over the age of 60 and 50% over the age of 80 and it is the leading cause of disability and weakness of the elderly, with severely impaired quality of life.

The same regenerative potential of skeletal muscle decreases with age and that decline is related to the activity of satellite cells. The reduction of the function of satellite cells, as a source for new myofibers, can be connected to the decrease of the reservoir of these cells; in fact, it has been demonstrated that the abundance of satellite cells, residing in myofibers, tends to decrease with increasing age.

STEM CELLS AS A PERSPECTIVE FOR THE FUTURE MUSCLE REGENERATION

Cell therapy represents an exciting approach to the regeneration of muscle tissue suffering from degenerative diseases. This method aims to the use of stem cells that can differentiate into skeletal muscle, restoring the functionality.

Thanks to their characteristics and their myogenic potential, satellite cells are considered among the candidates for the therapeutic approach in the treatment of muscular dystrophies. Pioneeristic studies in literature have demonstrated that intramuscular injection of these cells, in the mouse with Duchenne dystrophy, leads to the fusion of these existing fibers and an

effective production of dystrophin. Nevertheless, in the '80s, several clinical trials have failed for different reasons, including poor survival and migration of donor cells after transplantation and the rejection of these cells, due to the immune response of the patients.

In view of this, today, many preclinical studies, are aimed at increasing the survival, proliferation and differentiation of stem cells after transplantation. Moreover, although of potential benefit for particular myopathies, such as Duchenne muscular dystrophy, the practical use of satellite cells is limited primarily due to the poor availability, since the cells must be obtained from the muscle tissue of the patient, and the decrease of their self-regenerative potential with increasing age.

An alternative resource to satellite cells is represented by mesenchymal stem cells derived from adipose tissue. It has been shown that stem cells derived from adipose tissue can differentiate into different cell types in vitro (adipogenic, osteogenic, chondrogenic) and the commitment of these cells can be determined by simple addition in the culture medium, specific differentiating factors. This cell population can, therefore, represents a promising resource of stem cells for many strategies for tissue regeneration, thanks to the fact of being available in large quantities, readily obtainable by simple liposuction, with minimal morbidity and discomfort for the patient. All these features combined with the fact that you have a high capacity for ex vivo expansion and a very low immunogenic behavior, mean that stem cells derived from adipose tissue are an important tool in cell therapy for the treatment of degenerative muscle diseases.

MUSCLE AS ENDOCRINE ORGAN

Skeletal muscle is mainly characterized by its mechanical function, which is essential to maintain posture, movement and breathing, whose processes depend on the contraction of muscle fibers. However, the skeletal muscle is not only a component of our locomotor system. Recent evidence has, in fact, identified the skeletal muscle as a secretory organ, able to produce and release, into the circulation, several proteins, called myokines, in response to the contraction, which can affect the metabolism and the function of muscle tissue itself but also other organs or tissues, such as liver, brain, adipose tissue, the cardiovascular system.

Skeletal muscle, following a request by the body, can adapt by increasing the size of muscle fibers and it is no surprising if many of the factors secreted by muscle contraction, act

regulating hypertrophy and muscle repair. In this sense, the myokines allow the muscle to regulate the growth and its regeneration, for example, as a consequent adaptation to exercise. The myokines can thus adjust the skeletal myogenesis in numerous ways, including the process that involves the activation and migration of satellite cells, the subsequent proliferation and differentiation into myoblasts and their fusion in muscle fibers.

So far, only for few identified myokines the biological effect has been studied. It is however clear that such substances can serve in future as potential therapeutic targets for the treatment of those diseases that affect growth and muscle regeneration and may provide an explanation of the fact that a regular exercise delays the aging process. It is expected that we can ameliorate symptoms of the degenerative muscle diseases and sarcopenia associated with aging. In fact, it could be conceivable that physical inactivity can lead to an alteration or impairment of the production of myokines and/or to a resistance of their effects, thus explaining as the lack of physical activity increases the risk of an entire number of diseases, such as cancer, cardiovascular disease, type 2 diabetes, dementia and osteoporosis.

Characteristics of myokines

- cytokines produced, expressed and released by muscle fibers
- can have an autocrine effect (the substance acts on the cell that produced it), paracrine (the substance acts on the surrounding cells), or endocrine (the substance acts on the distant cells in a similar manner to hormones)
- several hundred secreted myokines, not yet identified, constitute the secretome of the muscle cell
- between the identified myokines are myostatin, interleukin -6 (IL-6), interleukin-7 (IL-7), leukemia inhibitory factor (LIF), insulin-like growth factor-1 (IGF-1), fibroblast growth factor-2 (FGF-2), follistatin-like-1 (FSTL-1) and irisin
- can balance and counteract the effect of adipokines (substances produced by adipose tissue)
- may mediate the protective effect of muscular exercise for those diseases associated with inactive lifestyles

MUSKENDO PROJECT

MUsclecrinology: characterization of SKeletal uscle as a target organ and a key player in the ENDOcrine-metabolic

The **MUSKENDO** project, recently funded by the Ministry of Education, University and Research, is addressing as a primary objective the endocrinology of the skeletal muscle, which will be characterized both as an endocrine organ and as target organ for several hormones.

In vitro analysis will characterize the specific human muscular endocrine apparatus in terms of hormonal receptors, second messengers and secreted substances in primary cultures of mature myocytes. The effects on muscle secretome (mRNA, microRNA, proteins) of several hormones, such as thyroid hormones, insulin, GH, will be analysed. Adipocytes, a promising source of stem cells for multiple tissue engineering strategies, will be used to develop models of skeletal myogenesis where the plasticity of the endocrine apparatus will be further assessed. Electrophysiologic studies will also be undertaken given the importance of the coordinated activity of different modulating signals and ionic channels in myogenesis. Once established the main involved pathways, the responses to specific hormones in terms of proliferation, differentiation and secreted proteins will be tested.

Ex vivo analyses will be carried out in skeletal muscle biopsies obtained in healthy individuals (mainly during elective surgery), obese patients suffering from sarcopenic obesity and in patients suffering from hypothyroidism or hyperthyroidism. The expression profile (transcriptome, miRNoma) will be analysed and the specific endocrine expression signature will be assessed (expression of hormonal receptors, specific responses elicited by hormones in terms of second messengers, secreted cytokines/hormones also in primary cultures obtained from these muscular specimens. In this context, the expression of modified ataxia telangiectasia mutated (ATM) protein, a kinase which is known to be expressed in skeletal muscle and involved in insulin and IGF-1 signaling, will be assessed in primary cultures of human myocytes in physiological and pathological conditions.

(Expanding with various links in future)

TIPS TO MAINTAIN GOOD MUSCLE MASS

To maintain muscle mass is essential to support the metabolism, the immune system, the physical strength and the vitality. The muscle mass can be preserved by an appropriate ratio between synthesis and degradation of proteins in muscle cells.

A balanced nutrition, including adequate proteins intake and regular exercise, especially endurance sports, can help to maintain or to rebuild muscle mass and promotes an active and independent life.

Two millennia ago, Hippocrates observed that "walking is the best medicine for men". Since then, the benefits of physical activity on health are universally recognized and the actual reduction in the risk of death and increased longevity have been well documented in the literature.