Closing report of the OTKA-K111941 grant

Period: 01.01.2015 – 11.30.2017.

Title of the project: The role of macrophage PPARg in muscle regeneration

A project magyar címe: A PPARg magreceptor szerepe izomregenerációban

The aim of this project:

Hypothesis

The nuclear hormone receptor, PPARg regulates muscle macrophage gene expression and via this muscle regeneration

Specific aims:

1. Detailed characterization of the phenotype of macrophages in the absence of PPARg during muscle regeneration.
2. Identification of PPARg target genes using gene expression profiling.
3. Characterization of the contribution of PPARg regulated genes to muscle regeneration.

As the first step in our studies and in order to study the role of myelod cell, primarily macropjhages we have set up an experimentla systém involving bone marrow transpantation (BMT), irradiation, shielding and in vivo imaging.

Skeletal muscle regeneration is a complex interplay between various cell types including

invading macrophages. Their recruitment to damaged tissues upon acute sterile injuries is

necessary for clearance of necrotic debris and for coordination of tissue regeneration. This

highly dynamic process is characterized by an in situ transition of infiltrating monocytes from

an inflammatory (Ly6Chigh) to a repair (Ly6Clow)macrophage phenotype. The importance of the macrophage phenotypic shift and the cross-talk of the local muscle tissue with the infiltrating macrophages during tissue regeneration upon injury are not fully understood and their study lacks adequate methodology. In the first set of studies using an acute sterile skeletal muscle injury model combined with irradiation, bone marrow transplantation and in vivo imaging, we could show that preserved muscle

integrity and cell composition prior to the injury is necessary for the repair macrophage phenotypic transition and subsequently for proper and complete tissue regeneration. Importantly, by using a model of in vivo ablation of PAX7 positive cells, we show that this radiosensitive skeletal muscle progenitor pool contributes to macrophage phenotypic transition following acute sterile muscle injury. In addition, local muscle tissue radioprotection by lead shielding during irradiation preserves normal macrophage transition dynamics and subsequently muscle tissue regeneration. Taken together, our data suggest the existence of amore extensive and reciprocal cross-talk between muscle tissue compartments, including satellite cells, and infiltrating myeloid cells upon tissue damage. These interactions shape the macrophage in situ phenotypic shift, which is indispensable for normal muscle tissue repair dynamics.

Key points of this study include:

The in situ phenotypic switch of macrophages is delayed in acute injury following irradiation.

The combination of bone marrow transplantation and local muscle radiation protection allows

for the identification of a myeloid cell contribution to tissue repair.

PET-MRI allows monitoring of myeloid cell invasion and metabolism.

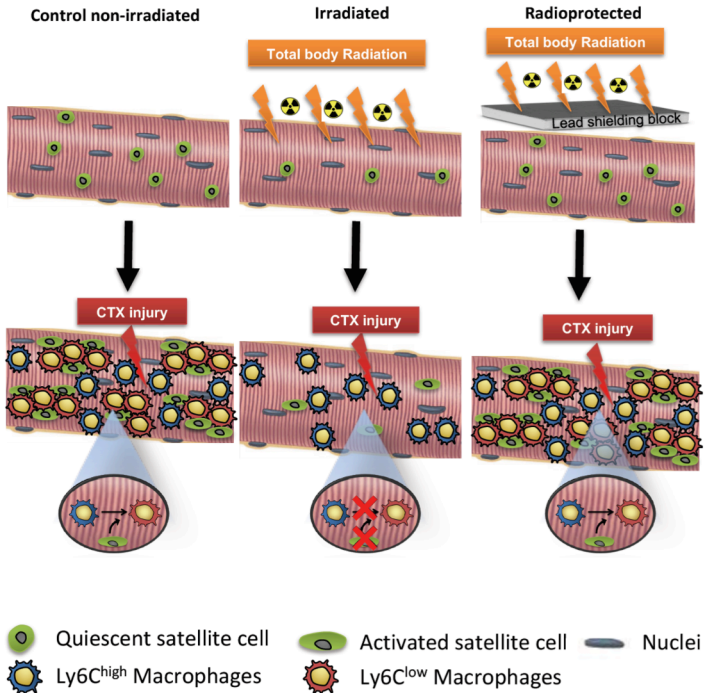
Altered cellular composition prior to acute sterile injury affects the in situ phenotypic transition of invading myeloid cells to repair macrophages.

There is reciprocal intercellular communication between localmuscle cell compartments, such as PAX7 positive cells, and recruited macrophages during skeletal muscle regeneration.

This work is summarized on figure 1 and was published in Patsalos et al J. Physiology (2017)

Figure 1.

Satellite cells affect MF transition from inflammatory to repair phenotype, which is critical for normal muscle tissue repair dynamics.



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In the second set of studies and addressing Specific Aim 1 we have carried out a comprehensive transcriptomic analysis.

Macrophage gene expression determines phagocyte responses and effector functions. Macrophage plasticity has been mainly addressed in in vitro models that do not account for the environmental complexity observed in vivo. In this study, we showed that microarray gene expression profiling revealed a highly dynamic landscape of transcriptomic changes of Ly6CposCX3CR1lo and Ly6CnegCX3CR1hi macrophage populations during skeletal muscle regeneration after a sterile damage. Systematic gene expression analysis revealed that the time elapsed, much more than Ly6C status, was correlated with the largest differential gene expression, indicating that the time course of inflammation was the predominant driving force of macrophage gene expression. Moreover, Ly6Cpos/Ly6Cneg subsets could not have been aligned to canonical M1/M2 profiles. Instead, a combination of analyses suggested the existence of four main features of muscle-derived macrophages specifying important steps of regeneration:

1) infiltrating Ly6Cpos macrophages expressed acute-phase proteins and exhibited an

inflammatory profile independent of IFN-g, making them damage-associated macrophages;
2) metabolic changes of macrophages, characterized by a decreased glycolysis and an increased tricarboxylic acid cycle/oxidative pathway, preceded the switch to and sustained their anti-inflammatory profile;
3) Ly6Cneg macrophages, originating from skewed Ly6Cpos cells, actively proliferated; and
4) later on, restorative Ly6Cneg macrophages were characterized by a novel profile, indicative of secretion of molecules involved in intercellular communications,
notably matrix-related molecules. These results show the highly dynamic nature of the macrophage response at the molecular level after an acute tissue injury and subsequent repair, and associate a specific signature of macrophages to predictive specialized functions of macrophages at each step of tissue injury/repair.

Utilizing the developed technology and identifying candidate regulators from the expression profiling prompted us to examine the role of macrophage PPARg and address Specific Aims 2 and 3 in the next set of studies.
Tissue regeneration requires inflammatory and reparatory activity of macrophages. Macrophages detect and eliminate the damaged tissue and subsequently promote regeneration. This dichotomy requires the switch of effector functions of macrophages coordinated with other cell types inside the injured tissue. The gene regulatory events supporting the sensory and effector functions of macrophages involved in tissue repair are not well understood. In these studies we could convincingly show that the lipid activated transcription factor, PPARg is required for proper skeletal muscle regeneration, acting in repair macrophages. PPARg controls the expression of the transforming growth factor-b (TGF-b) family member, GDF3, which in turn regulates the restoration of skeletal muscle integrity by promoting muscle progenitor cell fusion. This work establishes PPARg as a required metabolic sensor and transcriptional regulator of repair macrophages. Moreover, this work also establishes GDF3 as a secreted extrinsic effector protein acting on myoblasts and serving as an exclusively macrophagederived regeneration factor in tissue repair.

Key points of this study are:

Macrophage PPARg is required for skeletal muscle regeneration

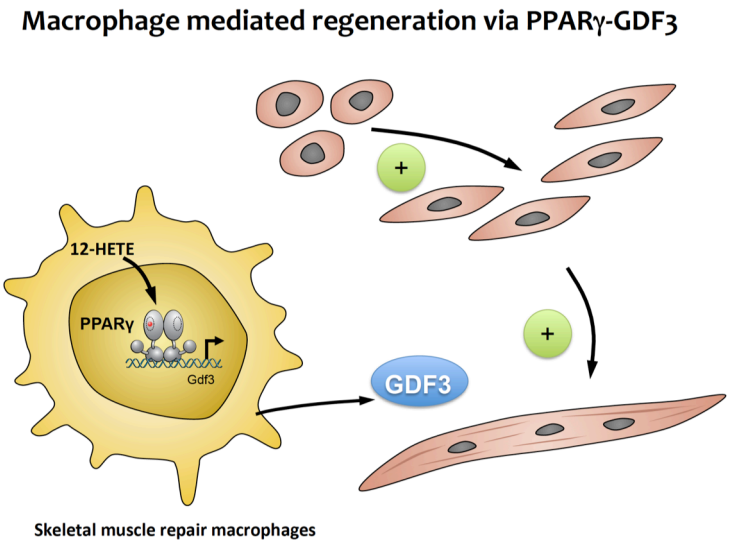
PPARg regulates GDF3 in muscle infiltrating Ly6C_ repair macrophages

The Gdf3 locus has multiple PPARg :RXR heterodimer-bound active enhancers

GDF3 regulates muscle regeneration and enhances primary myoblast fusion

This is depicted on Figure 2 and was published in Varga et al Immunity (2016).

Figure 2.



Based on these observations and findings and as a follow up of the published studies in the last part of the project we have generated new hypothesis

This new hypothesis is that In vivo GDF3 administration abrogates aging related muscle regeneration delay following acute sterile injury.

We tested this using an aging model in mice. Tissue regeneration is a highly coordinated process with sequential events including immune cell infiltration, clearance of damaged tissues and regrowth of the tissue. Aging has a clear negative impact on this process, however whether the immune cells per se are contributing to the decline in the body`s ability to regenerate tissues with aging is not clearly understood. In these new studies we set out to characterize the dynamics of macrophage infiltration and their functional contribution to muscle regeneration by comparing young and aging animals upon acute sterile injury. Injured muscle of

old mice showed markedly elevated number of macrophages, with a predominance for Ly6C^{high} pro-inflammatory macrophages and a lower ratio of the Ly6C^{low} repair macrophages. Of interest, a the recently identified repair cytokine, growth differentiation factor-3 (GDF3) was markedly downregulated in injured muscle of old relative to young mice. Supplementation of recombinant GDF3 in aged mice ameliorated the inefficient regenerative response. Together, these results uncover a distinctive macrophage secreted GDF3-mediated mechanism of muscle regeneration during aging and suggest that in vivo administration of GDF3 could be an effective therapeutic approach for muscle injury in elderly people.

This is depicted on Figure 3.

Figure 3.