Wnt4 acts as a differentiation factor on C2C12 myoblasts and satellite cells

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WNT4 ACTS AS A DIFFERENTIATION FACTOR ON C2C12 MYOBLASTS AND SATELLITE CELLS

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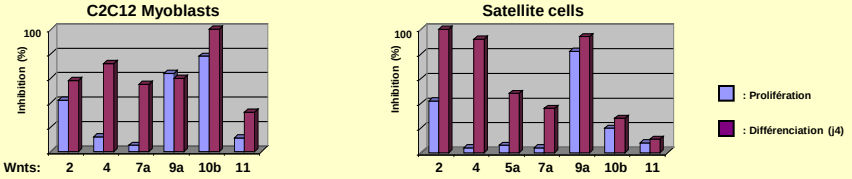
PRESENTATION: The Wnt family of proteins, which are found throughout the animal kingdom, are responsible for the determinism and development of many tissues during embryogenesis. The Wnt system is also a post-natal regulator of many physiological and physiopathological processes such as angiogenesis, adipogenesis, cell growth and death, pathological development of the nervous system, tumorisation… With the exception of somite development during early myogenesis, the involment of Wnts in muscle homeostasis is less well known. They play an important role in the myogenic specification of certain adult stem cells, in terminal differentiation and the balance between fast and slow fibres in the chicken, and during post-natal growth and muscle hypertrophy in myostatin knock-out mice. The aim of this study is to characterize the role of Wnt proteins in muscle homeostasis, state of equilibrium between anabolism (hypertrophy) and catabolism (atrophy).

I. Expression patterns of Wnt genes during C2C12 myoblasts and satellite cells differentiation:



Methods: Mice satellite cells were isolated from the whole muscles of the paw. RNA was extracted using Ademtech total RNA isolation reagent. Analysis of Wnt genes was made by quantitative real-time RT-PCR using Syber Green on Biorad Mini opticon. GAPDH was used as an invariant control.

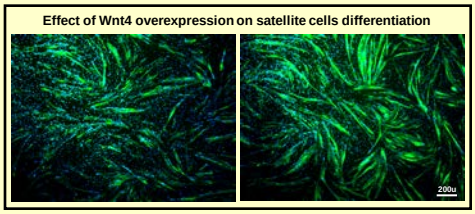
Results: Only a few Wnts are expressed in proliferative and differentiated states. In C2C12 myoblasts and satellite cells, Wnt4 and Wnt7a are strongly induced during differentiation (8- and 11-fold for C2C12 myoblasts and 50- and 9-fold for satellite cells, respectively). Wnt4 being highly expressed in both differentiated cell types, we have limited our study to this factor.

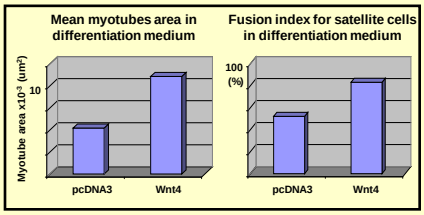
II. Wnt4 and myogenesis:

⇨ Wnt4 overexpression

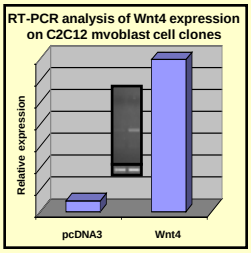
Methods: Stable C2C12 cell clones were generated by transfection with pcDNA3.1 expression vector containing Wnt4 (Wnt4) or lacking Wnt-4 (pcDNA3). Clones were selected by G418 selection.
Satellites cells were transiently transfected with pcDNA3.1-based vector containing or lacking Wnt4 by using Dreamfect transfection reagent.
Quantification of mRNA encoding for Wnt4 and myogenic factors was made by using real-time RT-PCR with GAPDH as invariant control.
Immunolabeling was achieved with anti-myosin antibody and nuclei were stained with DAPI reagent. Myotubes mean area and fusion index were measured by densitometric analysis using ImageJ software.

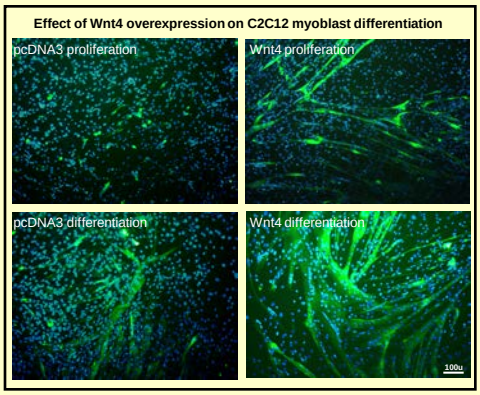
Satellite cells:

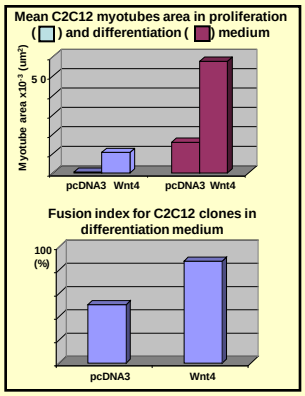


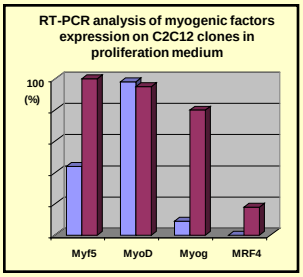


C2C12 Myoblasts:



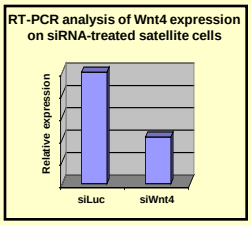


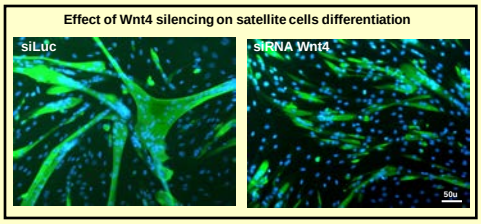


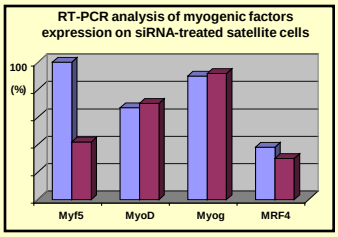


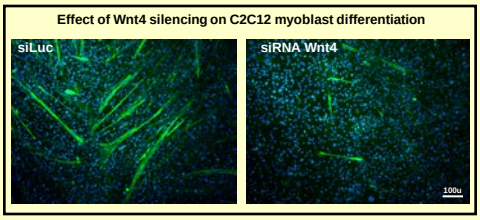
⇨ Wnt4 silencing

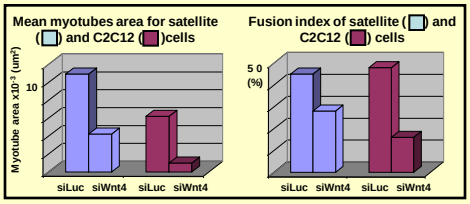
Methods: C2C12 myoblasts and mouse satellite cells were transfected with in vitro transcribed Luciferase (siLuc) or Wnt4 (siWnt4) siRNA by using Dreamfect transfection reagent. Myogenic factors and Wnt4 expression were measured by semi-quantitative RT-PCR, using GAPDH as invariant. Immunolabeling was achieved with anti-myosin antibody and nuclei were stained with DAPI reagent. Myotubes mean area and fusion index were measured by densitometric analysis using ImageJ software.











Results: