

Adiposity - GRIM1/NIR

Target for the Treatment of Disturbances of Skeletal Muscle and Fat Cell Differentiation

Albert-Ludwigs-Universität Freiburg



UNI
FREIBURG

Technology

The therapeutic modification of the function of GRIM1/NIR may counteract premature muscle degeneration or actively intervene in processes which build up muscle. Furthermore GRIM1/NIR shows a sub-nuclear relocalization during adipocyte differentiation. This technology further relates to antibodies which specifically bind to an epitope of GRIM1/NIR. Monoclonal humanized antibodies of this type can be employed in therapy. An alternative area of use of these antibodies is diagnosis. Furthermore this invention relates to a method for identifying substances which influence the biological function of GRIM1/NIR. It is possible with this test system to identify substances which interact with the GRIM1/NIR polypeptide and inhibit or enhance the biological function of GRIM1/NIR.

Innovation

- Humanized monoclonal antibodies for therapy or
- polyclonal antibodies for diagnostic of
 - degenerative muscle disorders
 - disturbances in fat cell differentiations

Application

- Degenerative muscle disorders
- Controlling muscle atrophy in the aging man
- Controlling of fat cell differentiations, e.g. in case of adipositas

Market Potential

- Muscular atrophy affects a major number of elderly people. Muscular atrophy decreases quality of life as the sufferer becomes unable to perform certain tasks or worsen the risks of accidents while performing those.
- 30% of the US population and 20% of the European population are considered to be obese.

Responsible Scientist

Prof. Dr. Roland Schüle

Dept. of Molecular Gynecology

Branch

Pharma

Patent Status

Patent No.:
EP 1485412 B1,
US 7981623 B2

Filed (PRD) March 20th 2002

Reference Number

ZEE20010713b

Status: Aug-11

Contact

Campus Technologies Freiburg GmbH | Stefan-Meier-Str. 8 | D-79104 Freiburg
Email: Harald.App@campus-technologies.de
Tel: +49 (0)761 203-5020
Fax: +49 (0)761 203-5021





Control of adipogenesis by the signal-regulated INHAT-repressor NIR

Natalia Kunowska, Na Yin, and Roland Schüle

Central Clinical Research, Freiburg University Medical Center, Germany

Summary:

To initiate the functional characterization of NIR during development and organogenesis, we applied a tandem affinity purification (TAP) strategy combined with mass spectrometry (MALDI-TOF) to identify NIR-interacting partners such as the tumour suppressor p53 (Hublitz et al., 2005). Association of p53 and NIR was verified in vitro and in vivo. Upon recruitment by p53, NIR represses transcription of both p53-dependent reporters and endogenous target genes. Knockdown of NIR by RNA interference significantly enhances acetylation of histones at p53-regulated promoters. Moreover, p53-dependent apoptosis is robustly increased upon depletion of NIR. In summary, our findings describe NIR as a novel INHAT that plays an important role in the control of p53 function.

The TAP approach revealed also several other NIR-interacting proteins such as FAD24, neuroguidin, peter pan, and PES1, that are described to play important role in adipogenesis (Tominaga et al., 2004; Jung et al., 2006; Suarez-Huerta et al., 2000; Lapik et al., 2004). Importantly, by bioinformatic data mining of the yeast (Gavin et al., 2006) and *C. elegans* proteome (Zhong et al., 2006), respectively we uncovered that Noc2p, the yeast and worm homologs of NIR, form a complex with the respective homologs of FAD24, Neuroguidin, peter pan, and PES1. Thus, this NIR associated complex in evolutionary conserved. In addition, MacKay et al. 2003 showed that in *C. elegans* lpd-2 (neuroguidin homolog), lpd-6 (peter pan homolog), and lpd-7 (Pes1 homolog) are required for fat storage. Finally, a genome-wide RNAi analysis of *C. elegans* revealed that knockdown of the NIR homolog Pro-2 results in a "fat-reduced" phenotype (Ashrafi et al., 2003). The physical interaction between FAD24, neuroguidin, peter pan, PES1, and NIR was confirmed in vitro and in vivo. Moreover, we could show direct interaction between NIR and GSK3 β , a key regulator in adipocyte differentiation (Ross et al., 2000). In vitro, NIR is a substrate for GSK3 β -mediated phosphorylation, implicating NIR function in Wnt signalling. Finally, miRNA-mediated knockdown of NIR severely impairs adipogenesis in mammalian cells. In summary, these data suggest a physiological role of NIR in adipogenesis.

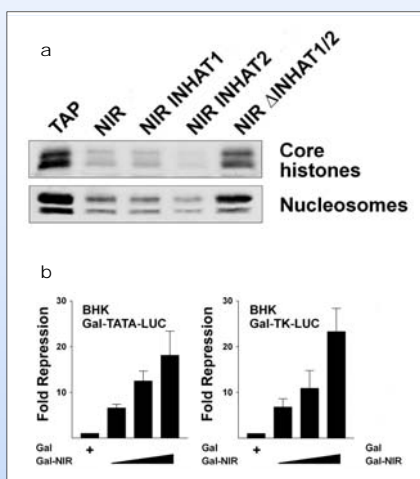


Figure 2: NIR is a novel INHAT repressor
a) NIR is a functional INHAT. Full-length NIR and both INHAT-domains block acetylation by p300. b) NIR is a potent transcriptional repressor. Gal-NIR represses transcription of minimal or complex Gal-dependent reporters in a dose-dependent manner (Hublitz et al. Genes Dev. 19, 2005).

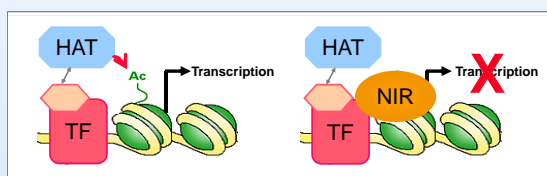


Figure 1: NIR is an INHAT repressor
Upon recruitment to chromatin by a transcription factor (TF), NIR interacts with histone tails and blocks acetylation by histone acetyltransferases (HATs), which results in transcriptional repression.

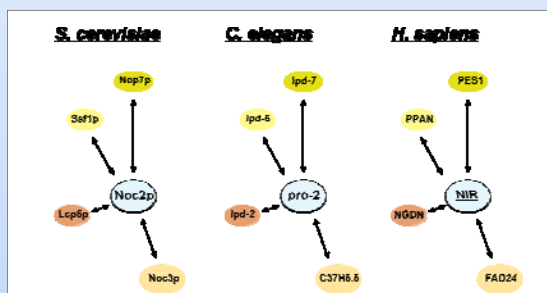


Figure 5: The interaction of NIR with PPAN, PES1, NGDN and FAD24 is evolutionary conserved (Gavin et al. 440 Nature, 2006; Zhong et al. Science 311, 2006).

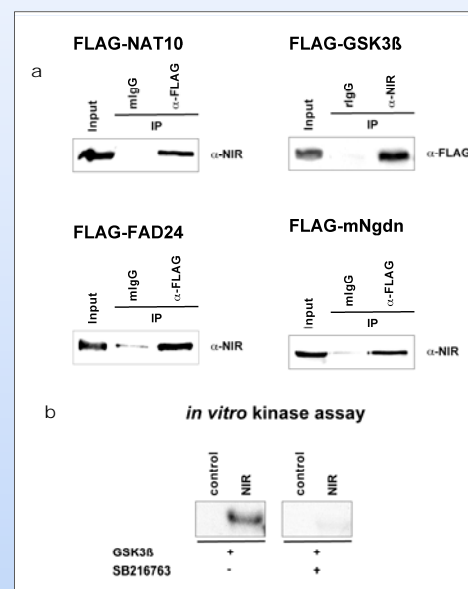


Figure 4: NIR assembles with NAT10, GSK3 β , PPAN, PES1, NGDN and FAD24
a) NIR co-immunoprecipitates with Nat10, GSK3 β , neuroguidin and FAD24. b) NIR is a direct target of GSK3 β in vitro. The phosphorylation of NIR by GSK3 β is inhibited by the GSK3 β inhibitor SB216763.

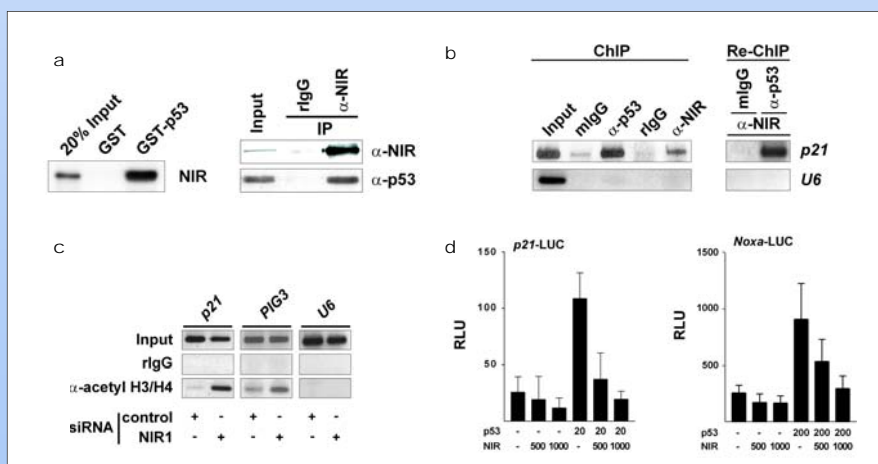


Figure 3: NIR regulates p53 function
a) NIR interacts with p53 in vitro and in vivo as demonstrated by GST pull-down and IP respectively. b) NIR and p53 assemble at p53 target promoters in vivo. c) Knockdown of NIR leads to hyperacetylation of histone tails at p53 dependent promoters. d) NIR represses transcription of p53-dependent promoters in transient transfections assays. (Hublitz et al. Genes Dev. 19, 2005).

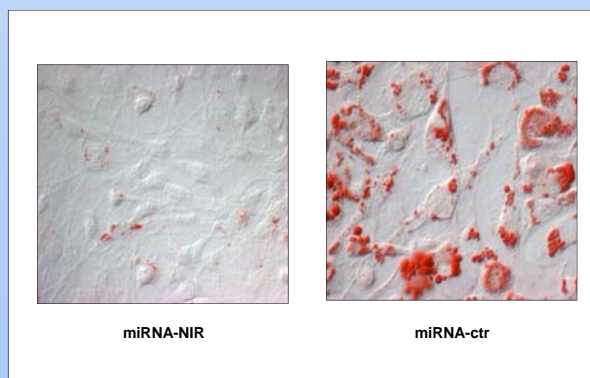


Figure 6: NIR is a critical regulator of adipocyte differentiation in vitro.
NIR depletion results in severe impairment of 3T3-L1 adipogenesis, as visualised by Oil-Red staining.

Publications:

- Hublitz et al. Genes Dev. 19 (2005)