Ronan Crépin, David Gentien, Angeline Duché, Audrey Rapinat, Cecile Reyes, Fariba Némati, Gérald Massonnet, Didier Decaudin, Selma Djender, Sandrine Moutel, Klervi Desrumeaux, Nathalie Cassoux, Sophie Piperno-Neumann, Sebastian Amigorena, Franck Perez, Sergio Roman Roman, Ario de Marco (2017 Feb 1)

**Nanobodies against surface biomarkers enable the analysis of tumor genetic heterogeneity in uveal melanoma Patient Derived Xenografts.**

*Pigment cell & melanoma research*: DOI: [10.1111/pcmr.12577](https://doi.org/10.1111/pcmr.12577)

**Summary**

Monoclonal antibodies specific for biomarkers expressed on the surface of uveal melanoma (UM) cells would simplify the immune-capture and genomic characterization of heterogeneous tumor cells originated from patient derived xenografts (PDXs). Antibodies against four independent tumor antigens were isolated by panning a nanobody synthetic library. Such antibodies enabled flow-cytometry-based sorting of distinct cell sub-populations from UM PDXs and to analyze their genomic features. The complexity and specificity of the biochemical and genomic biomarker combinations mirrored the UM tumor polyclonality. The data showed that MUC18 is highly and universally displayed at the surface of UM cells with different genetic background and consequently represents a reliable pan-biomarker for their identification and purification. In contrast, the other three biomarkers were detected in very variable combinations in UM PDX cells. The availability of the identified nanobodies will be instrumental in developing clone-specific drug evaluation and rational clinical strategies based on accurate genomic profiling. This article is protected by copyright. All rights reserved.

Hélène de Forges, Antoine Pilon, Isabelle Cantaloube, Antoine Pallandre, Anne-Marie Haghiri-Gosnet, Franck Perez, Christian Poüs (2016 Dec 6)

**Localized Mechanical Stress Promotes Microtubule Rescue.**


**Summary**

Microtubule dynamics rely on the properties of tubulin and are regulated by microtubule-associated proteins. GTP-tubulin assembles into hollow polymers, which can depolymerize upon GTP hydrolysis. Depolymerizing microtubules may stop shrinking and resume growth. Such rescues are regulated by microtubule-associated proteins like CLIP-170 and the CLASPs [1, 2]. Microtubule domains prone to rescues contain discrete regions (previously termed “GTP islands”) that retain a GTP-tubulin-like conformation in the main body of the microtubule [3]. However, the exact nature of these domains and the mechanisms controlling their occurrence and distribution are largely unknown. Here we show that collisions between growing microtubules and mechanical obstacles (including other
microtubules) in vitro result in the higher abundance of GTP-like islands in stressed microtubule regions. Furthermore, these islands were found to be efficiently generated by both lateral contacts and mechanical constraints applied to the main body of the microtubules. They were also particularly prominent where shifts in the number of protofilaments occur in the microtubule lattice. GTP-like islands and rescues frequently co-occurred at microtubule intersections in vitro and in living cells, both in crossing and in crossed microtubules. We also observed that CLIP-170 recognizes GTP-like islands in vivo and is retained at microtubule crossings. Therefore, we propose that rescues occur via a two-stage mechanism: (1) lattice defects determine potential rescue-promoting islands in the microtubule structure, and (2) CLIP-170 detects these islands to stimulate microtubule rescue. Our results reveal the interplay between rescue-promoting factors and microtubule architecture and organization to control microtubule dynamics.

Sandra Scharaw, Murat Iskar, Alessandro Ori, Gaelle Boncompain, Vibor Laketa, Ina Poser, Emma Lundberg, Franck Perez, Martin Beck, Peer Bork, Rainer Pepperkok (2016 Nov 23)

The endosomal transcriptional regulator RNF11 integrates degradation and transport of EGFR.
The Journal of cell biology : 543-558

Summary

Stimulation of cells with epidermal growth factor (EGF) induces internalization and partial degradation of the EGF receptor (EGFR) by the endo-lysosomal pathway. For continuous cell functioning, EGFR plasma membrane levels are maintained by transporting newly synthesized EGFRs to the cell surface. The regulation of this process is largely unknown. In this study, we find that EGF stimulation specifically increases the transport efficiency of newly synthesized EGFRs from the endoplasmic reticulum to the plasma membrane. This coincides with an up-regulation of the inner coat protein complex II (COPII) components SEC23B, SEC24B, and SEC24D, which we show to be specifically required for EGFR transport. Up-regulation of these COPII components requires the transcriptional regulator RNF11, which localizes to early endosomes and appears additionally in the cell nucleus upon continuous EGF stimulation. Collectively, our work identifies a new regulatory mechanism that integrates the degradation and transport of EGFR in order to maintain its physiological levels at the plasma membrane.


NaLi-H1: A universal synthetic library of humanized nanobodies providing highly functional antibodies and intrabodies.
eLife : DOI : 10.7554/eLife.16228

Summary

In vitro selection of antibodies allows to obtain highly functional binders, rapidly and at lower
cost. Here, we describe the first fully synthetic phage display library of humanized llama single domain antibody (NaLi-H1: Nanobody Library Humanized 1). Based on a humanized synthetic single domain antibody (hs2dAb) scaffold optimized for intracellular stability, the highly diverse library provides high affinity binders without animal immunization. NaLi-H1 was screened following several selection schemes against various targets (Fluorescent proteins, actin, tubulin, p53, HP1). Conformation antibodies against active RHO GTPase were also obtained. Selected hs2dAb were used in various immunoassays and were often found to be functional intrabodies, enabling tracking or inhibition of endogenous targets. Functionalization of intrabodies allowed specific protein knockdown in living cells. Finally, direct selection against the surface of tumor cells produced hs2dAb directed against tumor-specific antigens further highlighting the potential use of this library for therapeutic applications.