Optically activating a cell signaling pathway using carbon nanotubes

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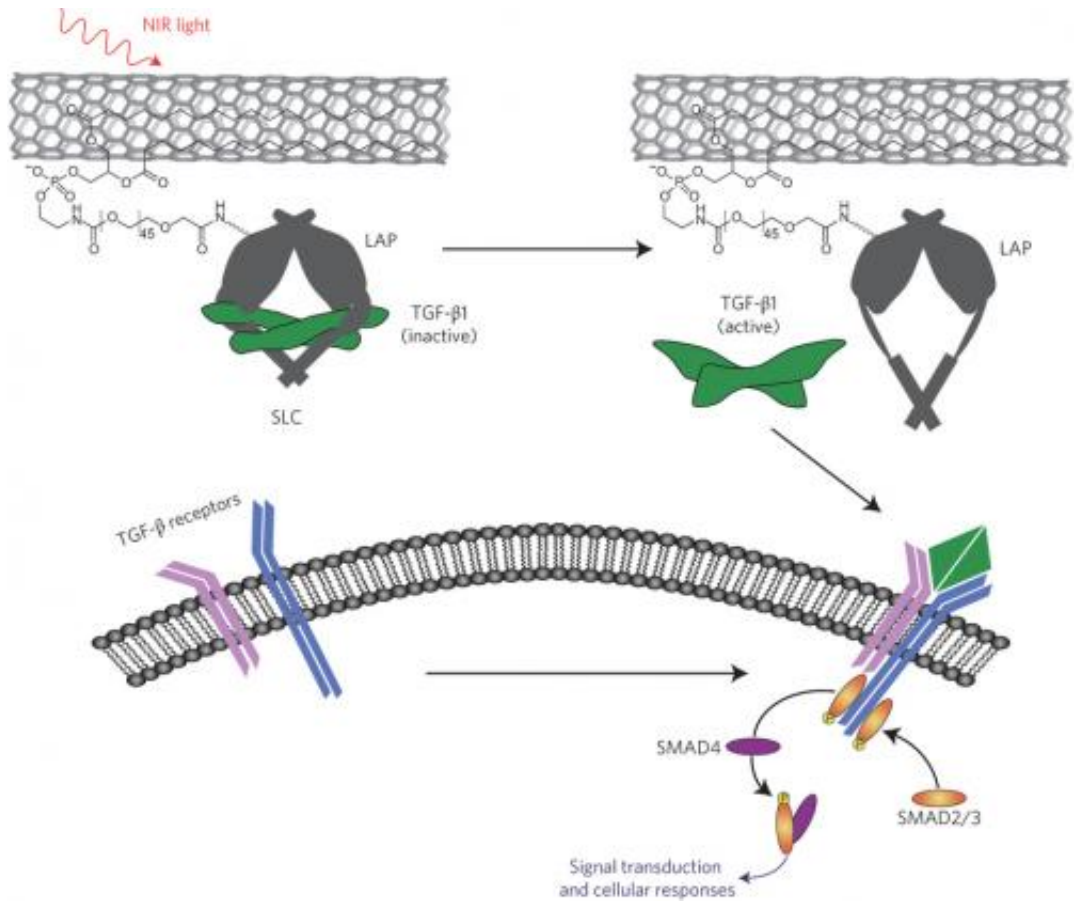

Schematic of carbon nanotube-assisted optical activation of TGF-β signalling by NIR light. SLC, the complex of TGF-β1 and LAP, is chemically conjugated on the SWCNT surface. NIR irradiation generates local heating near the nanotube surface, which disrupts the association between TGF-β1 and LAP and therefore releases TGF-β1. The released TGF-β1 becomes active and binds to TGF-β receptors on cell surfaces to trigger downstream signal transduction. Typically, R-SMAD (SMAD2/3) is phosphorylated by the activated receptors. The phospho-SMAD2/3 forms a complex with SMAD4. By translocating into the nucleus, the

complex regulates downstream gene expression and cellular responses. Credit: (c)2015 NPG, *Nature Nanotechnology*, doi:10.1038/nnano.2015.28

(Phys.org)—Our bodies have highly regulated and integrated systems. One such system, the TGF-β cell signaling pathway, regulates many developmental processes. A malfunctioning TGF-β pathway is implicated in many diseases, including cancer, developmental defects, and kidney disease. Typically this is due to a problem in the regulatory system that says when to activate TGF-β. Because it is involved in so many cellular processes, scientists are interested in finding ways to control TGF-β activation.

Professor Xiang Chen, graduate student Liang Lin and undergraduate student Ling Liu, along with a team of researchers from Peking University, Tsinghua University, and Harvard Medical School have found a way to activate TGF- β using near infrared (NIR) irradiation by combining the small latent complex (SLC) formed from TGF- β and its latency-associated peptides with a single-walled carbon nanotube (SWCNT). Their work, which investigates both in vitro and in vivo systems, is published in *Nature Nanotechnology*.

SWCNTs have two responses when irradiated with NIR light. They will respond by heating, or and they will produce reactive oxygen species. Prior studies have shown that these effects can be used to destroy cancer cells and denature proteins when they are associated with a SWCNT. Lin and Liu investigated whether these same effects can be used to control the TGF-β signaling pathway by conjugating SLC onto the SWCNT. The idea is to irradiate the SWCNT-SLC complex so that it would release active TGF-β.

The authors were particularly interested in using NIR to spurn the

activation of TGF-β, by exploiting SWCNT's response to NIR. NIR is also biologically gentler than ultraviolet and visible irradiation. Also, NIR is better at penetrating through surfaces than UV-vis, including skin, and is less likely to cause photodamage to cells.

Lin and Liu et al. conducted three experiments in which they confirmed that TGF-β can be activated by NIR irradiation of the SWCNT-SLC complex, and they also confirmed that TGF-β maintains its biological activity both in cellular and mouse models.

The first experiment involved conducting in vitro studies to test their supposition that SLC and be conjugated onto SWCNT using known chemical conjugation methods, and that TGF-β would be released upon irradiation by NIR. They confirmed, using SDS-PAGE, that SLC was conjugated onto the SWCNT using their procedure.

The next step in their in vitro studies tested whether irradiation by NIR successfully released TGF-β (Note: In this study, they used porcine LAP in complex with TGF-β1 for their SLC protein). They irradiated a solution of the complex using a 980 nm NIR laser for five minutes, and then used centrifugal filtration to separate the complex from TGF-β. The supernatant was mostly the SWCNT complex with the latency peptide and the flow-through contained TGF-β, confirming that TGF-β was successfully released.

Lin and Liu et al. then determined that the released TGF-β maintained its biological activity, which is binding to cell-surface receptors and activating the cellular signal pathway. The tests that NIR-released TGF-β induced the activity of a TGF-β-responsive reporter as strong as pure TGF-β indicated that TGF-β does maintain its biological activity after being activated via NIR irradiation of SWCNT-SLC.

Finally, in an effort to optimize their experimental conditions before

moving to in vivo studies, various NIR wavelengths were tested to see if they obtained similar results. They found that NIR wavelengths at 808 nm, 915 nm, and 1,064 nm all effectively activated TGF-β, allowing for some versatility when using this technique in living systems.

The second experiment involved taking the optimized results from the in vitro studies and applying them to live cells. SWCNT-SLC was incubated with cells and then irradiated with NIR for five minutes. Phosphorylation of TGF-β signaling mediator SMAD2 and enhanced transcription of three endogenous TGF-β/SMAD pathway target genes showed that TGF-β was successfully activated and bound to TGF-β receptors to initiate the signaling pathway.

Since cellular studies indicated that the SWCNT-SLC was relatively stable and non-toxic, the last experiment involved preliminary mouse studies in which NMuMG cells that were pre-incubated with SWCNT-SLC were implanted under the flank of a mouse. The site was irradiated by a 980 nm laser through the skin. Tests showed that TGF-β was activated after NIR irradiation, but was not activated in the control group in which the cells were implanted but not irradiated.

Overall, this method shows that TGF-β can be optically activated by conjugating its complex with SWCNT. Additionally, this method could be used as a general strategy for optically controlling the activation of certain biomolecules.

More information: Carbon nanotube-assisted optical activation of TGF-[beta] signaling by near-infrared light, *Nature Nanotechnology*, DOI: 10.1038/nnano.2015.28

Abstract
Receptor-mediated signal transduction modulates complex cellular behaviours such as cell growth, migration and differentiation. Although

photoactivatable proteins have emerged as a powerful tool for controlling molecular interactions and signalling cascades at precise times and spaces using light, many of these light-sensitive proteins are activated by ultraviolent or visible light, which has limited tissue penetration. Here, we report a single-walled carbon nanotube (SWCNT)-assisted approach that enables near-infrared light-triggered activation of transforming growth factor β (TGF-β) signal transduction, an important signalling pathway in embryonic development and cancer progression. The protein complex of TGF-β and its latency-associated peptide is conjugated onto SWCNTs, where TGF-β is inactive. Upon near-infrared irradiation, TGF-β is released through the photothermal effect of SWCNTs and becomes active. The released TGF-β activates downstream signal transduction in live cells and modulates cellular behaviours. Furthermore, preliminary studies show that the method can be used to mediate TGF-β signalling in living mice.