Synthesis of a photocaged tamoxifen for light-dependent activation of Cre-ER recombinase-driven gene modification.

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Public Summary:
The ability to permanently mark cells and track their development, called lineage tracing, has revolutionized developmental biology. A chemical known as Cre can cause changes in the genes (i.e., genetic recombination) in a cell so that the cell starts to produce proteins that can be distinguished under a microscope or by a cell sorting device. For example, Cre can turn on a gene the makes a cell and all of its offspring (i.e., cells it produces by cell division) express, for example, a green or a red fluorescent protein. Labeling of cells with these colored proteins can also be made specific to one type of tissue. This is done by placing the Cre enzyme that turns on a color next to a gene that is turned on only in certain tissues. For example, by restricting Cre expression to blood vessels, researchers found that cells within the blood were also marked by the reporter, suggesting that blood vessels can produce blood cells. Furthermore, Cre can be fused to an estrogen receptor (ER), so that the color labeling of cells is only activated when the ER is bound, for example by estrogen or estrogen-like molecules, such as tamoxifen or hydroxytamoxifen. Exposure of cells to hydroxytamoxifen will allow Cre-ER marking of cells only during the time window that hydroxytamoxifen is active (about one day), thus creating a single wave of cell marking. This provides an extra level of temporal control of cell marking. Thus, researchers using the same blood vessel controller for Cre-ER expression showed that the marking of blood cells occurred only when tamoxifen was given early in embryonic development, indicating that only embryonic blood vessels can produce blood cells. Despite the creation of Cre and Cre-ER mediated cell marking systems, they are unable to restrict marking to specific locations, such as the right side of an organ or the surface of a tissue. In this study, we addressed this problem by synthesizing a “photocaged” tamoxifen molecule. “Photocaging” renders the tamoxifen inactive, unless it is “uncaged” by the appropriate wavelength of light—in this case, ultraviolet light. We validated the photocaged tamoxifen using a Cre-ER cell line that initially expresses a red fluorescent protein, but when Cre is active will permanently switch to green fluorescent protein. We discovered that a one minute exposure to UV was sufficient to uncage the tamoxifen and induce the red/green switch, and a 5 minute UV exposure could induce a near-maximum level of uncaging. Further characterization revealed that the caged form of tamoxifen could rapidly enter cells and be uncaged later, paving the way for future studies by which caged tamoxifen could be injected into an animal, and a specific region of the animal exposed to UV light to activate cell marking only in that region. Our work adds spatial control of reporter expression to the temporal and tissue-specific control currently available.

Scientific Abstract:
We report the design of a water-soluble, quaternized tamoxifen photoprobe and demonstrate its application in light-controlled induction of green fluorescent protein expression via a Cre-ER recombinase system.