Light-Activated Gene Therapy, New Selective Therapies for Disease*

The development of transgenic mice that temporally and spatially control gene expression has dramatically advanced our understanding of biological processes. Technological advances have enhanced the resolution of mechanistic insights. Current technologies have been developed to allow the temporal control of gene expression within hours and up to months. Spatial resolution in mammalian systems has been at the level of the organ, using tissue-specific promoters. The development of caged ponasterone and its application to transgenics has allowed a new level of resolution, moving to the single-cell level of gene regulation and excision.

Knock-out and knock-in animal models are commonly employed to assess the biological role of specific proteins in the context of a multicellular organism. However, expression of individual genes is a temporal- and spatial- (i.e., tissue-) specific phenomenon that can influence both normal and abnormal biological processes. The use of temporally and spatially regulated transgenics is essential to understand the biology of genes that play a nonredundant role in development. Many of the genes implicated in cancer onset and progression also play an important role in development of the mammary gland (ERa, Stat5a, NFkB, Tcf, cyclin D1) (*1*). Inducible gene expression systems have been engineered so that alterations in expression can be triggered in the adult animal (*2–4*). A variety of constructs have been described, including systems in which the transgene is activated by heat shock, interferon, glucocorticoids, tetracycline, and isopropyl β thiogalactoside (*1*). Unfortunately, many of these systems display unfavorable properties, such as activator toxicity, activator-independent expression, endogenous gene activation, or low levels of transgene induction.

The cloning of the receptor for ecdysteroids led to the subsequent development of an ecdysteroid-inducible expression system that displays low basal activity with high inducibility (*4*). Ecdysone, an insect molting hormone, is not toxic and does not affect mammalian physiology (*5–8*). Transgenic mice were developed encoding a chimeric receptor for the ecdysone receptor, which regulated inducible expression of a β-galactosidase transgene in response to an injection of ecdysone (*4–8*). A limitation of the system at that time included the lack of bioactive material for in vivo studies. We screened botanical sources to identify a highly bioactive ecdysteroid, ponasterone A (*9*). We purified this plant steroid and developed sustained release pellets allowing application to transgenic mice for long-term in vivo studies (*11*). The system was also applied to allow regulatable gene expression in a controlled manner in the liver of transgenic mice using ecdysone-inducible viral delivery systems (*10*). The system was developed in transgenic mice in which tissue-specific expression was targeted to the mammary gland using a mammary gland–specific promoter. Sustained expression was maintained through ponasterone pellets (*11*).

Because several genes implicated in cancer onset and progression also contribute to breast development, it has proven difficult to fully evaluate the role of these genes in normal biology of the breast. The cyclin D1 gene has been implicated in mammary gland tumorigenesis, but the gene is also required for normal mammary gland development. In our recent studies we have developed ponasterone-inducible transgenics to inactivate the expression of cyclin D1 in the mature, fully developed mammary gland. By creating inducible cyclin D1 antisense mice targeted to the mammary gland we have determined the normal function of cyclin D1 in the mammary gland in vivo. The normal function of cyclin D1 in the mammary gland includes regulation of cellular migration, metabolism, and mitochondrial function. These studies, in turn, allowed the demonstration of the role for this gene in vivo as a regulator of cytosolic glycolysis by controlling gene expression in vivo in real time in live animals (*10*).

Although tissue-specific gene activation is feasible, the initiation of protein expression in small cell populations within specific tissue microenvironments is not. The design, synthesis, and evaluation of an ecdysone-based derivative that furnishes spatially discrete gene activation in a multicellular setting were subsequently developed (*12*). In previous studies photoremovable agents were used to render signal-transducing proteins and inhibitors inert until activated by light (*13–15*). In addition, light-activatable forms of DNA, RNA, and estrogen have been prepared and their ability to alter or promote protein expression described (*16–18*). We reported the preparation of an inert ecdysone derivative that can be spatially unleashed with light. Acylation at one or more of the free hydroxyl moieties generates analogs with little or no biological activity. We showed that exposure of cultured cells to the bioactive ecdysone generates a nearly 90-fold induction of luciferase. By contrast, the caged analog showed little detectable activation. A 1-minute photolysis of cells induced a dramatic enhancement of luciferase formation, with 70% of the expression displayed by the bioactive species. This is consistent with our observation that a 1-minute photolysis time window converts approximately 70% of the caged derivative into its bioactive counterpart.

*This presentation summarizes research carried out and reported by the author with Matthew Casimiro (Kimmel Cancer Center, Thomas Jefferson University; Philadelphia, PA) and Christopher Albanese, PhD, (Lombardi Comprehensive Cancer Center, Georgetown University Medical Center; Washington, DC).

Refinements are being made to apply this system to animal models. First, photoactivation furnishes only transient protein expression, thereby limiting the applicability of this approach to short-term biological phenomena. However, it should be possible to permanently alter gene expression profiles with a single treatment of light by coupling the light-inducible ecdysteroid strategy with the well established *Cre/loxP* recombinase system. Second, the *ortho*-nitrobenzyl moiety is removed with relatively short-wavelength (300–360 nm) light. The poor tissue penetrating properties of the latter precludes ready access to deeply interred tissue microenvironments. By contrast, multiphoton technology furnishes significantly deeper tissue penetration. A photolabile-protecting group containing a large, 2-photon absorbance cross-section has been introduced as a caging agent (*19*). These studies are currently in progress in the context of animal models.

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Rational Design for Peptide Drugs

The evolution of targeted macromolecules for molecular imaging with radiolabels transitioned from the highly specific but biologically long-lived monoclonal antibodies (mAbs) in blood resulting in images with low signal-to-noise ratio (SNR). The large size of mAbs (~145 kDa for IgG) (*1*) slows their diffusion into tissue, thus hindering their utility as imaging probes—at least for nuclear imaging, because of the hepatic metabolism of mAbs. The imaging requirement for low nonspecific signal from background necessitates rapid clearance and excretion of the labeled macromolecule probe from the blood to urine via the kidneys. The targeting macromolecule and labeling approach with radioactivity, fluorophores, MR-active metals, radioopaque atoms for CT, chelators, and linkers all may affect the biodistribution and excretory routes of a macromolecular probe.

Two possible alternatives to labeled mAbs using macromolecules with optimal biodistribution and excretory routes include (a) encapsulating the label into a particle, and (b) attaching multiple labels onto a macromolecular scaffold (*2*). In the first instance, a number of different macromolecular platforms, including polymers, proteins, dendrimers, liposomes, and ultrasmall superparamagnetic iron oxide particles, have been studied as carriers for imaging labels. In the second instance, multiple labels are attached to the backbone of a macromolecule (*3,4*). Preclinical studies of macromolecular carriers have established trends in routes and rates clearance with respect to agent size and charge, which depend on glomerular filtration, particularly over the ~30–50 kDa range (*5,6*).

The current discussion focuses on peptides targeting receptors for optical, PET, SPECT, MR, and CT imaging,

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