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Optogenetics: A new tool for cancer investigation and treatment

Siamak Alizadeh[1,2], Abolghasem Esmaeili[1], Jaleh Barar[2], Yadollah Omidi[3*]

[1]Department of Cell and Molecular Biology and Microbiology, Faculty of Biological Science and Technology, University of Isfahan, Isfahan, Iran
[2]Research Center for Pharmaceutical Nanotechnology, Biomedicine Institute, Tabriz University of Medical Sciences, Tabriz, Iran
[3]Department of Pharmaceutical Sciences, College of Pharmacy, Nova Southeastern University, Fort Lauderdale, Florida 33328, USA

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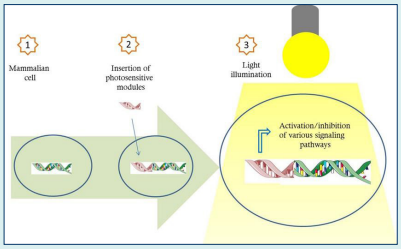
Abstract



Despite the progress made in the diagnosis and treatment of cancer, it has remained the second cause of death in industrial countries. Cancer is a complex multifaceted disease with unique genomic and proteomic hallmarks. Optogenetics is a biological approach, in which the light-sensitive protein modules in combination with effector proteins that trigger reversibly fundamental cell functions without producing a long-term effect. The technology was first used to address some key issues in neurology. Later on, it was also used for other diseases such as cancer. In the case of cancer, there exist several signaling pathways with key proteins that are involved in the initiation and/or progression of cancer. Such aberrantly expressed proteins and the related signaling pathways need to be carefully investigated in terms of cancer diagnosis and treatment, which can be managed with optogenetic tools. Notably, optogenetics systems offer some advantages compared to the traditional methods, including spatial-temporal control of protein or gene expression, cost-effective and fewer off-target side effects, and reversibility potential. Such noticeable features make this technology a unique drug-free approach for diagnosis and treatment of cancer. It can be used to control tumor cells, which is a favorable technique to investigate the heterogeneous and complex features of cancerous cells. Remarkably, optogenetics approaches can provide us with outstanding tool to extend our understanding of how cells perceive, respond, and behave in meeting with complex signals, particularly in terms of cancer evasion from the anticancer immune system functions.

Introduction

There are many treatment methods for cancer, including chemotherapy, surgery, radiotherapy, hormone therapy, and biological therapy. However most of them associate with harmful impacts on the healthy cells/tissue of the body, imposing a collective undesirable side effects in the patient under such treatment courses.[1-3] In the verge of emerging new cancer treatment techniques era, optogenetics provided exciting opportunities for scientists with the ability of (i) spatial-temporal control of cell function and behavior, (ii) fewer off-target cytotoxicity, and (iii) simple application with no/trivial undesired impacts.[4] The optogenetics concept is based on the combination of genetic and optical techniques for the rapid and reversible control of precise events in specific cells or tissues.[5] Given that light is a low-cost entity and nearly harmless, if used correctly, it can be delivered to the cells or organs with different wavelengths and in a controlled manner.[6] Photosensitive modules can reversibly activate or deactivate gene expression or effector proteins without causing long-term adverse effects in contrary to the traditional genetic perturbation approaches (knockout/knockdown or mutagenesis) that disturb the spatiotemporal features of the signaling network forever.[7] Light-controlled gene expression systems have been recognized for several cell types such as mammalian cells, yeast, and bacteria. [8] Optogenetics approaches are used more than a decade in neuroscience. However, over the last few years, optogenetics lines have been expanded dramatically.[9]

Cells can be considered analog robots, with a complex array of sensors and actuators (e.g., cell signaling and biomolecular circuitry) that function among the cell's exterior and interior.[10] Therefore, various numbers of

*Corresponding author: Yadollah Omidi, Email: yomidi@nova.edu

signaling pathway effectors, which are crucial for cancer expansion and progression and play an important role in cell fate and functions, became traceable for the management by light.[11] Considering this fact, a range of variants of optically activated signaling pathway initiators (e.g., Rho A, Raf 1, and Rac) have been established and used to study the cellular events with extraordinary precision. Table 1 lists some slected signaling pathways established with photoactivatable proteins.[12]

On the other hand, there are various numbers of protein modules such as cell surface receptors, extracellular proteins, and intracellular kinases that have been detected to spatially assemble into higher-order complexes. All these proteins can be managed by different inducible elements even though such phenomena are still poorly understood.[10,19] Although chemical stimuli have been extensively used in cancer research, their applications have been limited by some drawbacks, including difficulties in eliminating the inducer and diffusion-based transportation.[20] Hence, replacing light with chemical inducers offers an ideal gene expression system due to its extraordinary tunability and spatiotemporal resolution and received considerable attention.[21,22] Different types of light-inducible gene expression systems have been developed recently in mammalian cells.[23] By the development of gene editing technologies, optogenetics can be used in combination with the light-sensitive systems for cancer research and therapy.[20] In view of this, an optimized CRISPR-Cas9-based light-inducible gene expression system was used in bladder cancer cell models using the tumor suppressor *p53* gene. Their result indicated effective inhibition of bladder cancer cell 5637 and UMUC-3 proliferation *in vitro.*[20] As well, a light-controlled caspase (the main regulator of programmed cell death) has been created by combining a photoactivatable protein, light/oxygen/voltage (LOV) domain to the apoptosis-executing domain of caspase-7. Under blue light induction, LOV domain undergoes conformational change and releases caspase-7 domain, triggering apoptosis within 1 hour.[24] Recently, a light-controllable variant of receptor tyrosine kinases (Opto-RTKs) family, which is fundamental for cancer evolution and angiogenesis, has been reported by different research groups. Considering the clinical significance of RTK inhibitors, Opto-RTKs offer unlimited potential in the oncology field.[16,25] To this end, Opto-RTK has been used successfully to study the epithelial-mesenchymal transition which is one of the principal mechanisms in the cancer cell metastasis initiation and promotion.[2] Furthermore, a light-activatable form of G protein-coupled receptor (GPCR) family that plays the most important role in cell survival and apoptosis and is often dysregulated in most cancerous cells has been developed and used successfully by scientists.[26] In addition, optogenetics tools have been recently extended to remotely control immune responses by modulating dendritic cell (DC) maturation, inflammasome activation, lymphocyte trafficking, and photoactivation of calcium channels and engineered chemokine receptors for antitumor immunity.[27] Design of light-switchable immune cells (opto-immunoengineering) allows selective activation of these cells only at the desired time and location (e.g., a tumor tissue), thereby reducing off-target special effects.[28] For instance, an optogenetic controllable T cell system has been developed based on melanopsin for hepatocellular carcinoma immunotherapy which accurately regulates the functions of T cells via a calcium-NFAT pathway in response to blue light illumination.[29] Aside from the mammalian cells, optogenetics tools can be used in cancer therapy in combination with beneficial probiotic or engineered bacterial species.[30,31] Indeed bacterial cells are intelligent biological entities, which can sense various environmental changes (e.g., glucose, pH, oxygen, light, temperature) and respond properly.[30,32] Recently light responsible plasmids such as pDawn and pMars have been developed and used successfully by scientists. These constructs can express and produce anticancer genes or agents in the appropriate host bacterial species under the blue and red light illumination respectively.[33-35] Likewise, optogenetics tools have extended cancer drug discovery and delivery approaches.[36] Different kinds of screens are applied to drug candidates before subjecting to the clinical studies. These processes include *in vitro* and cell-based assays, tissues and primary cells screenings, and *in vivo* organism scale trials.[37] Currently used biochemical and cell-based assays are not specific, cost-effective, and rapid as they require testing a large number of small molecules.[37] Moreover, the employment of invasive measurement devices (for single-cell electrical measurements) or chemical addition may interfere and alter cellular activity in many cellular assays.[14,38] Hence, the development of optogenetics tools that address appropriate targets with reduced cost and 'contactless' activation or inhibition

Table 1. Some signaling pathways established with photoactivatable proteins

Signaling pathway	Light-activatable protein	Activation/deactivation wavelength (nm)	Signaling protein	References
MAPK	LOV	Blue light	Ste5	[13]
Ras/ERK	PhyB–PIF6	650-750 nm s/s	SOScat	[14]
Raf/MEK/ERK	CRY2–CIBN	Blue light (~450–480 nm)	Raf1	[15]
Opto-RTKs	LOV	Blue light(~450–480 nm)	FGFR	[16]
Apoptosis	LOV	Blue light(~450–480 nm)	Caspase-7	[17]
PI3K	CRY2–CIB1	Blue light(~450–480 nm)	SH2 of p85a	[18]

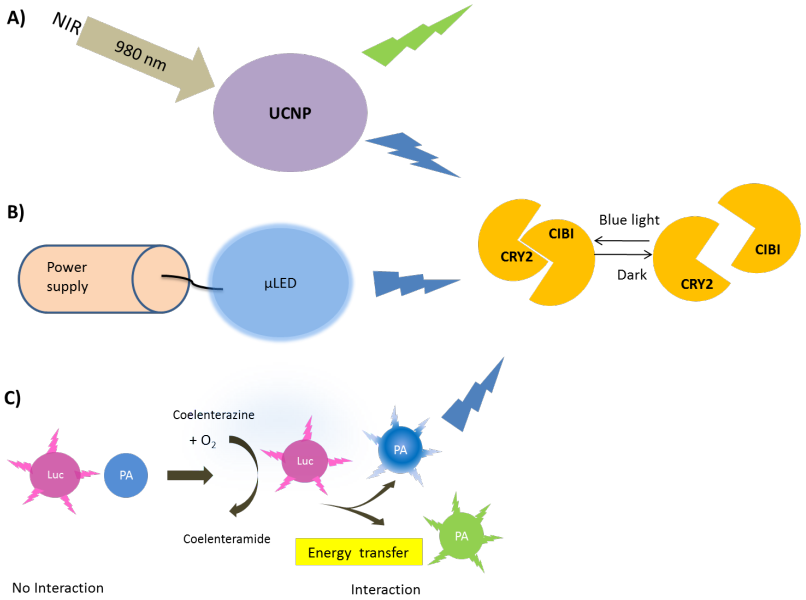


Fig 1. Different strategies for the *in vivo* application of optogenetics tools. (A) UCNPs which can convert near-infrared (NIR) light into visible and ultraviolet emission. (B) µLED implants that can provide wireless light power in deep layers of tissue by means of radio frequencies. (C) Luminopsin which can be created by fusing luciferases with the photoactivable protein can utilize exogenously provided chemical energy existed in small molecules to promote light-powered reactions. Luminopsin oxidases the high energy coelenterazine into the low energy product coelenteramide while transferring the light-energy to the photosensitive protein. UCNPs, upconverting nanoparticles; µLED, Micro LED; NIR, near-infrared; Luc, luciferases; PA, photoactivable protein; CRY2, cryptochrome 2.

of cellular activity promise to disclose new therapeutic codes.[14,39] A major challenge in the light-based activation process especially for *in vivo* applications has been the safety and penetration ability of the light deep into the tissue since visible lights are not able to penetrate more than several millimeters in tissue.[40] By the emerging advanced optogenetics toolkits, using implanted internal light sources such as fiber-light emitting diodes and optic light sources, this problem could be solved frequently.[41-43] Moreover, by using lanthanide-doped upconversion nanoparticles (UCNPs) which are capable to convert near-infrared (NIR) light into visible and ultraviolet emission, remote stimulation, and deep penetration into tissue are achievable.[44] These nanosacle transducers can up-convert several lower-energy photons into one high-energy photon which can activate nearly all current optogenetic constructs (e.g., CRY2, LOV2, and ChR2).[27,45] In this line, the NIR-stimulable optogenetic platform (named 'Opto-CRAC') in conjugation with NIR-to-blue emitting UCNPs, has been used effectively to improve the antitumor response with external NIR light in living animals.[46] Another strategy is the use of bioluminescence from luciferases as an alternative light source for the photoactivation of blue/green photosensitive proteins in a procedure that is named bioluminescence resonance energy transfer (BRET). In this method, luciferases are fused to microbial rhodopsin, giving rise to luminopsin which can convert high energy substrate coelenterazine into the low energy product coelenteramide. The light generated in this process is transferred simultaneously to the neighboring chromophore in a photosensitive protein (Fig. 1).[28, 29] Likewise, utilizing photosensitive proteins (PhyB–PIF and BphP1–PpsR2) that are excited by longer wavelength (red and NIR) and are able to penetrate tissue simply, could be an alternative approach.[28]

Nevertheless, some important parameters should be exploited experimentally, including (i) the reversibility and dynamics of the optogenetic tools, (ii) the endogenous availability of chromophores, and (iii) the precise degree of light with the particular wavelength and duration time.[6,47] For instance, a shorter wavelength (e.g., blue and UV lights) is appropriate for the surface of the skin, cell culture, and tissue explants whereas the red light (620–750 nm) and a part of the NIR light (750–1100 nm) are suitable for therapeutic application.[8] It should be noted that the long time exposure of the cell or tissue to the light might raise the temperature and cause tissue damage by severing alteration of cellular nucleic acids and proteins.[48] As well, optogenetic modules that need long exposure time might cause cell death by sustenance or over-activation of signaling pathways.[6] However, the development of more sensitive optogenetics modules with better kinetics could resolve this problem.[49] The optogenetic application could be extended by combining newly emerged genome-editing techniques with the photoactivatable domains for the precise editing of genome sequences.[50]

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Authors' contribution
SA, AB, JB, and YO gathered the data and drafted the manuscript. YO finalized the manuscript.

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