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Review

Trojan horse at cellular level for tumor gene therapies

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ABSTRACT

Among innovative strategies developed for cancer treatments, gene therapies stand of great interest despite their well-known limitations in targeting, delivery, toxicity or stability. The success of any given gene-therapy is highly dependent on the carrier efficiency. New approaches are often revisiting the mythic trojan horse concept to carry therapeutic nucleic acid, i.e. DNAs, RNAs or small interfering RNAs, to pathologic tumor site. Recent investigations are focusing on engineering carrying modalities to overtake the above limitations bringing new promise to cancer patients.
This review describes recent advances and perspectives for gene therapies devoted to tumor treatment, taking advantage of available knowledge in biotechnology and medicine.

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1. Introduction

Cancer remains a global health problem and a major cause of death worldwide. Statistical analysis published by the International Agency for Research on Cancer from the World Health Organization reveals that if the estimated trends continue, the incidence of all cancer cases will raise from 12.7 million new cases in 2008 to 21.2 million by 2030 (Bray et al., 2012).

Facing such an alarming disease progression, varieties of therapies have been developed but expected efficiency has still not been reached. Thus the need to develop innovative strategies is crucial.

Progress in the knowledge about tumor biology and molecular aspects of cancer has facilitated the design of new therapies aiming to overtake the limitations faced by research during the past decades. The main goal in anti-cancer approaches is to maximize efficacy of cancer treatments and to minimize systemic toxicity.

Abbreviations: AAV, adeno-associated viruses; Ad, adenovirus; CTGVT, cancer targeting gene-viro-therapy; EGFR, epidermal growth factor receptor; EPCs, endothelial precursor cells; GAOVT, gene armed oncolytic virus therapy; GCV, ganciclovir; HRE, hypoxia responsive element; HSV-TK, herpes simplex virus thymidine kinase; MDR1, multi drug resistance protein; MSCs, mesenchymal stem cells; NK, natural killers; OVs, oncolytic viruses; PAMAM, highly branched polyamidoamine; PEG, polyethylene glycol; PEI, poly(ethylenimine); PLL, poly(L-lysine); PU-PEI, polyurethane-short branch polyethylenimine; QD, quantum dots; SPION, superparamagnetic iron oxide nanoparticles; TERT, telomerase reverse transcriptase; THL, Trojan Horse Liposome; TK, thymidine kinase; TRAIL, tumor necrosis factor-related apoptosis-inducing ligand; VEGF, vascular endothelial growth factor.

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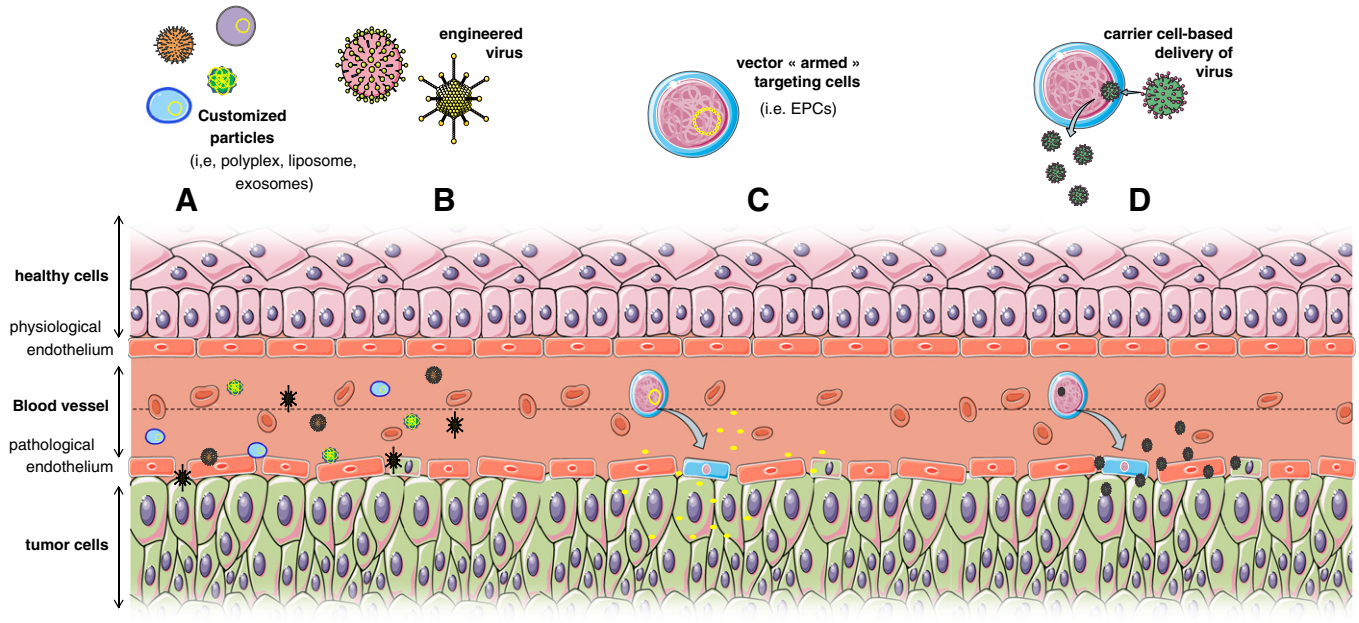

Fig. 1. An overview of various trojan horse strategies developed for cancer gene therapy. Schematic representation of approaches revisiting the trojan horse strategy for specific gene delivery to cancer cells. This double side scheme presents, in the upper part, a blood vessel in a physiological context, harboring a continuous and well organized endothelium. The lower part refers to a blood vessel in a pathological context of cancer where endothelium is disorganized, with tumor cells taking place in the vessel wall with endothelial cells. Such leaky and chaotic tumor vessels are supposed to give an access for therapy to cancer cells which can be targeted as well as tumor endothelium. In this aim, various trojan horses approaches have been developed. Numerous customized molecules such as polyplexes, liposomes or even exosomes (A) can be used and derivatized to reach preferentially the tumor area when injected in the blood stream, carrying either a DNA or interfering RNA (siRNA, miRNA). Viruses (B) can also be good carriers and bring an additional oncolytic activity. Homing cells like EPCs or MSCs (C) are used to target neoangiogenic sites. Following systemic application, engineered cells are recruited to the tumor environment where they will express the transgene, then acting on tumor cells. Targeted cells can be used for oncolytic viral particles production (D) upon recruitment in the tumor. To address therapies to cancer cells, these trojan horses combine regulatory "safety locks" to protect healthy cells. (Figure produced using Servier Medical Art).

Remaining a challenge, new approaches are now developed focusing on tumor microenvironment in addition to tumor cells themselves. This had led for example to the development of new methods for delivery of therapies by targeting the tumor-associated vasculature, providing thus promising antitumor effects with minimal systemic toxicity.

Gene therapy was born 50 years ago thanks to Dr. W. Szybalski and Dr. E. Szybalska's pioneer experiments (Szybalska and Szybalski, 1962) reporting the first gene transfer to mammalian cells. This field has been steadily developing and fast growing keeping its main focus on cancer. Moreover, a large number of anti-tumor strategies have been described. However the main obstacles to tissue and cell-specific gene delivery still remains. Biotechnology allows new strategies that improve and succeed to change the means of cancer treatments. Among elaborated approaches of gene therapies, the image of the "trojan horse" is largely used by strategies that combine, in the same engineered entity, a targeting unit and a specific drug/gene delivery system. Inspired by the Greek Mythology, the trojan horse was the source for the presented approaches and revisited for numerous applications. The trojan horse is an engineered specific tool in form of: a liposome, an exosome, a specialized cell or a modified virus in order to specifically reach the tumor site (Fig. 1). Hidden Odysseus's army symbolized the various therapeutic genes or interfering RNAs that are supposed to accurately target the gene of interest.

In gene therapy approaches, the final protein level modulation is obtained by exogenous DNA or mRNA delivery on the one hand, giving rise to specific protein expression. On the other hand, small RNAs (siRNAs, miRNAs) can be used to inhibit or modulate endogenous protein translation. To be efficient, these molecules have to resist the degradation in the circulation and further reach either cytoplasm (for RNA) or nucleus (for DNA) as well as escape endosomal degradation. As these active molecules are not predisposed to overcome physiological barriers to be delivered to the target cells, carriers are needed. Indeed, the latter should be able to facilitate nucleic acid stability in the circulation, intracellular delivery and, when required, import into the nucleus (Wang et al., 2012).

This review describes various approaches elaborated for tumor treatment addressed to the cell and sub-cellular levels and using the trojan horse tricky concept.

2. When the horse becomes a molecule

Non-viral gene delivery systems among which are cationic polymers such as poly(ethylenimine) (PEI)/poly(L-lysine) (PLL), dendrimers, carbon nanotubes, Superparamagnetic Iron Oxide Nanoparticles (SPION) or Quantum Dots (QD) are usually positively charged allowing compression of anionic nucleic acids in solution and interaction with negatively charged cell membranes. Thus, they may act as molecular trojan horses to allow gene delivery.

The most widely used are cationic polymers, PLL and PEI, that combine with DNA into particulate complex, called polyplex which enable the gene transfer into cells.

PEI allows polyplexes to efficiently escape the degradation within endosomes (Kichler et al., 2001). Polyurethane-short branch polyethylenimine (PU-PEI) or PEI are myristilated to help targeting brain tumor sites. They are used as a therapeutic-delivery vehicle in the treatment of glioblastoma either by delivering tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) in intracranial U87 glioblastoma-bearing mice (Li et al., 2011) or by delivering miRNAs (miR145) both *in vitro* in human glioblastoma-associated cancer stem like cells (CD 133^{+}) and *in vivo* in an orthotopic model induced by transplantation of the same cells in immunocompromised mice (Yang et al., 2012). Such polyplexes can be substituted by polyethylene glycol (PEG) to enhance their stability. PLL alone has poor transfection ability (Pouton et al., 1998). However PEG coating can increase both transfection efficacy and circulation half-life (Lee et al., 2002). Liu et al. in a recent study showed that PLL when associated with PEG and

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