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Self-focusing therapeutic gene delivery with intelligent gene vector swarms: Intra-swarm signalling through receptor transgene expression in targeted cells

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ABSTRACT

Background: Gene delivery *in vivo* that is tightly focused on the intended target cells is essential to maximize the benefits of gene therapy and to reduce unwanted side-effects. Cell surface markers are immediately available for probing by therapeutic gene vectors and are often used to direct gene transfer with these vectors to specific target cell populations. However, it is not unusual for the choice of available extra-cellular markers to be too scarce to provide a reliable definition of the desired therapeutically relevant set of target cells. Therefore, interrogation of intra-cellular determinants of cell-specificity, such as tissue-specific transcription factors, can be vital in order to provide detailed cell-guiding information to gene vector particles. An important improvement in cell-specific gene delivery can be achieved through auto-buildup in vector homing efficiency using intelligent 'self-focusing' of swarms of vector particles on target cells. Vector self-focusing was previously suggested to rely on the release of diffusible chemo-attractants after a successful target-specific hit by 'scout' vector particles.
Hypothesis: I hypothesize that intelligent self-focusing behaviour of swarms of cell-targeted therapeutic gene vectors can be accomplished without the employment of difficult-to-use diffusible chemo-attractants, instead relying on the intra-swarm signalling through cells expressing a non-diffusible extra-cellular receptor for the gene vectors. In the proposed model, cell-guiding information is gathered by the 'scout' gene vector particles, which: (1) attach to a variety of cells via a weakly binding (low affinity) receptor; (2) successfully facilitate gene transfer into these cells; (3) query intra-cellular determinants of cell-specificity with their transgene expression control elements and (4) direct the cell-specific biosynthesis of a vector-encoded strongly binding (high affinity) cell-surface receptor. Free members of the vector swarm loaded with therapeutic cargo are then attracted to and internalized into the intended target cells via the expressed cognate strongly binding extra-cellular receptor, causing escalation of gene transfer into these cells and increasing the copy number of the therapeutic gene expression modules. Such self-focusing swarms of gene vectors can be either homogeneous, with 'scout' and 'therapeutic' members of the swarm being structurally identical, or, alternatively, heterogeneous (split), with 'scout' and 'therapeutic' members of the swarm being structurally specialized.
Conclusions: It is hoped that the proposed self-focusing cell-targeted gene vector swarms with receptor-mediated intra-swarm signalling could be particularly effective in 'top-up' gene delivery scenarios, achieving high-level and sustained expression of therapeutic transgenes that are prone to shut-down through degradation and silencing. Crucially, in contrast to low-precision 'general location' vector guidance by diffusible chemo-attractants, ear-marking non-diffusible receptors can provide high-accuracy targeting of therapeutic vector particles to the specific cell, which has undergone a 'successful cell-specific hit' by a 'scout' vector particle. Opportunities for cell targeting could be expanded, since in the proposed model of self-focusing it could be possible to probe a broad selection of intra-cellular determinants of cell-specificity and not just to rely exclusively on extra-cellular markers of cell-specificity. By employing such self-focusing gene vectors for the improvement of cell-targeted delivery of therapeutic genes, e.g., in cancer therapy or gene addition therapy of recessive genetic diseases, it could be possible to broaden a leeway for the reduction of the vector load and, consequently, to minimize undesired vector cytotoxicity, immune reactions, and the risk of inadvertent genetic modification of germline cells in genetic treatment *in vivo*.

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1. Introduction

A gene vector is traditionally defined as a particle, which contains genetic material as a cargo and facilitates the transfer and establishment of this cargo in living cells. Gene therapy relies on gene vectors delivering DNA, mRNA, siRNA or other RNA species to cell populations requiring treatment. Gene delivery *in vivo* that is tightly focused on the intended target cells is essential to maximize the benefits of gene therapy and to reduce undesired side-effects. Thus, especially in repeated vector administrations, targeting helps to reduce vector load and, consequently, to minimize undesired immune reactions, vector cytotoxicity and the risk of inadvertent genetic modification of germline cells.

In general, there are three principles, which can be relied upon for the delivery and establishment of transgenes in particular populations of recipient cells: physical targeting (e.g., pinpoint injections or magnetic focusing), biological targeting (e.g., vector guidance through cell-specific cell-surface receptors) and combinatorial targeting, the latter exploiting sharper spatial profiles of gene delivery with multi-component (split) vector systems such as binary gene vectors [1]. These principles can be used cooperatively to achieve better control over the localization of gene transfer. Biological targeting of gene delivery with viral and synthetic vectors can be achieved through the decoration of gene vector particles with ligands (anti-receptors) binding cell-specific extra-cellular receptors, which guide gene transfer with vector particles to the desired cells [2–4]. In addition, biological targeting can rely on the use of vector-borne regulatory elements of cell-specific transgene expression (e.g., tissue-specific transcription enhancers or tissue-specific microRNAs), which function intra-cellularly and control the abundance of transgenic mRNA [5]. Development of cell-specific-ligand-based targeted gene vectors with high delivery efficiency and high homing efficiency depends on the availability of both suitable cell-specific extra-cellular receptors in intended target cells and appropriate vector-borne ligands, which are often designed through multiple rounds of protein engineering consuming considerable time, labour and finance [6]. Generation of efficient transgene-expression-based cell-targeted gene vectors is similarly cumbersome because of difficult-to-avoid tradeoffs between strength and cell-specificity of gene expression. Therefore, any new strategies to boost delivery and/or homing efficiencies of cell-targeted gene vectors are extremely desirable.

With the targeted drug delivery setting in mind, a vector system was proposed by P. Grancic and F. Stepanek [7], where the much needed enhancement in the homing efficiency of drug delivery was achieved through the 'crowding' of vector particles around target cells, induced by the release and spread of chemo-attractants by few 'scout' vector particles after these particles successfully reached their designated target cells. In this scenario, the cell-targeted drug vector particles behave as an intelligent swarm capable of auto-buildup in vector homing efficiency, that is, 'self-focusing', on target cells. In the above model of Grancic and Stepanek, all vector particles are loaded with both drug and chemo-attractant cargos; the resultant swarm is, therefore, homogeneous. There is an important class of heterogeneous swarms [8] and, undoubtedly, the model of P. Grancic and F. Stepanek [7] can be extended to consider self-focusing vector swarms with specialized drug-loaded 'therapeutic' and chemo-attractant-loaded 'scout' particles.

The key element for the intelligent behaviour of a swarm is a channel for the transmission of information from one swarm member to another [9]. Within the complex milieu of the human body, intra-swarm signalling through the release and spread of diffusing chemo-attractants is technically challenging and might not be the optimal 'pointing' strategy for targeted delivery of genetic medicines using therapeutic gene vector swarms. An alternative communication channel between the gene vector particles is required. Gene vectors occupy a unique position among drug delivery vectors, as their genetic cargo is a *bona fide* instruction message for the recipient cells. Thus, this message could be exploited and non-trivial involvement of recipient cells in intra-swarm communication is a comprehensible and attractive possibility for the generation of intelligent therapeutic swarms of genetic vehicles.

2. Hypothesis

I propose that intelligent self-focusing behaviour of swarms of cell-targeted therapeutic gene vectors can rely on intra-swarm signalling through target cells, where vector-borne transgene expression control elements (e.g., tissue-specific transcription enhancers) can be used to interrogate intracellular determinants of cell-specificity and to direct biosynthesis of strongly binding (high affinity) cognate receptors for these gene vectors. Thus, the 'scout' gene vector particles, which have attached to a variety of cells via a weakly binding (low affinity) receptor and successfully facilitated gene transfer into these cells, could transmit clear cell-specific homing signals to the rest of the vector swarm. The attraction of new vector particles to cells expressing a strongly binding receptor is expected to result in cell-specific escalation of therapeutic gene transfer. I hypothesize that such self-focusing gene vector swarms could be particularly effective for sustaining high-level therapeutic transgene expression in curative scenarios involving the prolonged circulation of gene vector particles, for example, in repeated vector administrations, which are used for 'topping-up' therapeutic transgene expression to compensate for gene silencing.

3. Evaluation of the hypothesis

3.1. Engineering of vectors with receptor-based self-focusing gene delivery

As cells propagate and differentiate during an individual's lifetime, they acquire distinctive tissue-specific and cell-specific features. Abnormal cells, such as cancer cells, also have unique molecular signatures. Cell-specificity can be viewed as a property, which is distributed throughout the live cell, and is defined, in part, by the extra-cellular determinants (cell surface markers) and, in part, by intracellular determinants (Fig. 1). Cell surface markers are directly available for interrogation in live cells and are often used for targeted guidance of gene vectors towards specific cell populations [2,6]. However, it is not unusual for the available extra-cellular markers to be too scarce to provide a reliable definition of the desired target cell populations. Thus, in many instances of cell targeting, it would be attractive to probe intracellular determinants of cell-specificity in order to provide exhaustive cell-guiding information to gene vector particles. One way to query intracellular determinants of cell-specificity is through epigenetic-state-dependent expression of transgenes [5]. To be available for the circulating gene vector particles, the results of this query should be transmitted to the cell surface, where they could be available to attract new vector particles. Therefore, cell-specifically expressed transgenes coding for extra-cellular markers, such as protein receptors for viral or nonviral vectors, can provide the required communication channel between the pioneer 'scout' vector particles, which first entered the cell, and the vector particles, which are still circulating.

Tissue-specific transcription enhancers responsive to specific bouquets of transcription factors could be a robust choice for the cell-specific gene expression control elements directing selective expression of the signalling receptor transgene in the targeted cell