ciency. (1). A muscle-specific eukaryotic gene expression plasmid, pSV40E/MCK-HIF1a, was constructed by integrating SV40-enhancer with MCK promoter to regulate HIF-1a gene expression. (2) *In vitro* and *in vivo* studies both indicated that, compared with the natural MCK promoter, the SV40E/MCK hybrid promoter significantly increased HIF-1a gene expression, while retaining a good muscle-cell specificity. Although less efficient than the nonspecific CMV promoter, the hybrid promoter provided more stable gene expression and represented a good compromise between transcriptional activity and muscle specificity. (3) *In vitro* biological effects of increasing HIF-1a gene expression were analyzed in myoblasts to evaluate the function of the muscle-specific gene expression system. Real-time PCR showed up-regulation of several critical angiogenic genes expression, such as VEGF, ANGPT-1, MMP-2 and SDF-1, which were previously demonstrated to facilitate new blood vessel formation and/or maturation. Transwell cell migration assay revealed that pSV40E/MCK-HIF1a transfected L6 cells could recruit progenitor cells derived from bone marrow and muscle tissue. These observations suggested the muscle-specific gene expression system may be useful for stimulating new blood vessel growth and maturation in ischemic limbs while restricting the therapeutic effect to muscle tissue. (4) When reporter gene was transferred into mice limb skeletal muscles, using various nonionic natural polymers, including hyaluronic acid, alginic acid and dextran, the formulated plasmid/polyer resulted in different levels of reporter gene expression, depending upon the type and concentration of the polymers. Some of them showed better performance than naked DNA and these results indicated that the pSV40E/MCK-HIF1a combined with a suitable nonionic polymer may provide a safe and efficient gene therapy system for treatment of limb ischemia.

doi:10.1016/j.drudis.2010.09.384

A34
Amphipathic CPPs upregulate Ca in cells' cytosol and induce lysosomal exocytosis

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Cell penetrating peptides (CPP) promote the uptake of different bioactive cargo molecules that makes the harnessing of CPPs a promising strategy for drug design and delivery. The translocation mechanism of CPPs into cells, however, has still remained elusive. Direct passage of peptides across the plasma membrane might interfere with its integrity and introduce disturbances. In our study we assessed how cells compensate the disturbances and which processes are induced in response to CPP uptake. Applying fluorometry, flow cytometry and fluorescence microscopy we demonstrate that the uptake of various CPPs enhances the calcium levels in Jurkat and HeLa cells' cytoplasm. The elevated cytoplasmic free calcium concentration evokes downstream effects of membrane repair response and lysosomal exocytosis. Our results indicate that ten of the most commonly used CPPs can be divided into three groups based on their interaction with plasma membrane, the induction of calcium influx, and downstream responses: (1) primary amphipathic CPPs (e.g. MAP, TP) that modulate plasma membrane integrity inducing influx of calcium ions into cells and activate membrane repair and lysosomal exocytosis starting, from low concentrations; (2) arginine-rich, secondary amphipathic, CPPs (e.g. Penetratin, pVEC) that induce changes in the intracellular calcium concentration or subsequent responses at relatively high concentrations and (3) non-amphipathic CPPs (e.g. Tat, Arg9) that do not evoke changes in the intracellular calcium concentration or subsequent responses even at high concentrations. Triggering of the plasma membrane repair response may help cells to recover by replacing the misorganized or membrane active CPPs containing plasma membrane regions, whereas non-amphipathic CPPs could infiltrate without subsequent cellular responses.

doi:10.1016/j.drudis.2010.09.385

A35
Cellular delivery of oligonucleotides by PepFect

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PepFect (PF) series of peptide based transfection reagents have been developed for the delivery of oligonucleotides (ON) and plasmids into cells. Some PFs are also capable of nuclear delivery of oligonucleotides, for example phosphorothioate 2′-O-methyl RNA oligomers translocate into nucleus and rescue the luciferase expression in the splicing redirection assay after coupling to PF. The optimal ratio of ON with PF for obtaining the functional complexes has been described earlier, but it is not known how such particles interact with the cell surface, enter cells, and reach nucleus. In order to characterize the oligonucleotide delivery by PFs, we labelled 2′-OMe ON with 1.4 nm Nanogold (NG) particles. The membrane interaction, uptake, and intracellular traffic of ON–NG after complexing with PFs were mapped by transmission electron microscopy to unravel their internalization mechanism. PFs pack the Nanogold-labelled ON into small (~200 nm) particles in solution. Smaller particles of ON–NG–PF complexes associate later to form bigger assemblies at the surface of HeLa cells and are taken up by cells in vesicles. The size, electron density and regularity of ON–NG–PF containing structures vary largely depending on the PepFect and its concentration. In cells the majority of the complexes locate in the endosomal/lysosomal vesicles after four hours of incubation. However, the vesicles often have a discontinuous membrane and the Nanogold-labelled oligonucleotides can be found in the cytosol. In addition, with the help of some PFs, the oligonucleotides also reach the cell nucleus. Our results demonstrate that non-covalent complexes of Nanogold-labelled oligonucleotides with PepFects form particles that concentrate at the cell surface and enter cells by endocytotic mechanism. The finding that oligonucleotides have reached nucleus suggests that ON–PF complexes could induce the destabilization of endosomal mem-

branes, followed by the escape of ON from vesicles and translocation into nucleus. Our electron microscopy results are in line with data published earlier regarding the redirection of splicing with oligonucleotides delivered into cells by PFs.

doi:10.1016/j.drudis.2010.09.386

A36
Live-cell imaging and single-particle tracking of polyplex internalization

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Systemic delivery of therapeutic genes for gene therapy or cancer gene therapy requires gene vectors that overcome several barriers. The vector has to enable tissue-selective delivery, internalize efficiently and finally release its cargo reliably within the target cell. Tissue specificity and enhanced internalization can be achieved by cell-specific ligands that bind to certain surface markers that are upregulated in, for example, solid cancers. Functionalization with pH-sensitive and redox-sensitive linkers or polymers allows the vector to 'sense' external stimuli that will trigger their activation in temporally and spatially controlled manner. We investigate the uptake of targeted and untargeted polymeric gene vectors (polyplexes) by highly sensitive fluorescence microscopic methods on a single cell level [1]. The epidermal growth factor receptor (EGFR) is overexpressed on a high percentage of human carcinomas and is therefore an attractive therapeutic target for tissue-specific targeting by non-viral vectors in cancer gene therapy. Comparing uptake kinetics and internalization dynamics, single particle tracking in combination with quenching experiments revealed typical three-phase dynamics of the uptake process independent of targeting. Phase I was characterized by slow, actin-cytoskeleton-mediated movement of the particles with drift and included the internalization process. During phase II, particles displayed increased velocities with confined and anomalous diffusion in the cytoplasm. Phase III was characterized by fast active transport along microtubules. Targeting of polyplexes for receptor-mediated endocytosis by the EGF receptor resulted in shortening of phase I and strongly accelerated internalization. Targeted as well as untargeted particles were transported in early endosomes marked by Rab5–GFP and accumulated in late endosomes marked by Rab9–GFP. The endosomal release dynamics of polyplexes consisting of DNA condensed with the cationic polymers linear polyethyleneimine (LPEI), poly-(L)-lysine (PLL) or poly-(D)-lysine (PDL) were studied by photochemical release in living cells [2]. Using double-labeled polyplexes, DNA and polymer were imaged simultaneously by dual-color fluorescence microscopy. Our results demonstrate that the characteristics of the cationic polymer significantly influence the release behavior of the polyplexes. For LPEI/DNA particles, LPEI quickly spread throughout the cytosol, whereas DNA was released slowly and remained immobile thereafter. In the case of PLL particles, both DNA and polymer showed quick release. PDL particles remained condensed upon photosensitizer activation.

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doi:10.1016/j.drudis.2010.09.387

A38
Vascular endothelium remodeling in human African trypanosomiasis

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Molecule movement into the central nervous system (CNS) is restricted by the blood–brain barrier (BBB) and the blood–cerebrospinal fluid (CSF) barrier. Human African trypanosomiasis (HAT) or sleeping sickness, caused by the parasites, *Trypanosoma brucei* (T.b.) gambiense or *T.b. rhodesiense*, is fatal if untreated. The first disease stage is associated with trypanosome proliferation in the periphery. The second stage is when the parasites reach the CNS. HAT treatment is stage specific with drugs, which are assumed to cross the BBB, used to treat CNS stage disease. Since the treatment of CNS-stage HAT is more toxic than that of early-stage, it is vital to stage HAT [1]. Staging requires a CSF sample. Lumbar puncture under field conditions is difficult and invasive. Improved tests for staging HAT are required [2]. Our studies have established that *T.b. brucei* crosses the murine blood–CNS interfaces at ~day 11 post-infection (p.i.) and the animals died at day 37.9 ± 1.23. At day 7, 14 and 21 p.i. no loss of barrier integrity was measurable using the inert tracer, [^{14}C]sucrose (342 Da; radius 4.6 Å), nor was there any endothelium remodeling (including transporter up/downregulation) as measured with eflornithine, pentamidine or nifurtimox [3,4]. BBB, but not choroid plexus, dysfunction, occurred at days 28 and 35 p.i. with resultant increases in [^{14}C]sucrose space [3,4]. Suramin (1429 Da) brain distribution increased at day 35 p.i., suggesting considerable BBB breakdown as this molecule is highly albumin (60 kDa; radius 35.5 Å) bound [4,5]. Furthermore, the increased [^{14}C]sucrose association with the endothelial cell at day 35 p.i. compared to the non-infected and other infected time groups suggested an increase in vesicular trafficking [3]. This loss of integrity may be a sign of terminal disease. However, perhaps there was an earlier loss of blood–CNS barrier integrity (possibly when the parasites entered the CNS) that was not measurable using [^{14}C]sucrose (an inert tracer with smaller molecular dimensions being needed) and/or this was a reversible process that was undetectable at the times studied. Furthermore, endocytosis may be a sensitive marker of endothelium remodeling. The characterization of vesicular expression in a murine model of HAT may be the first step towards vesicle targeted staging strategies. Overall understanding blood–CNS barriers breakdown in HAT could contribute to the development of therapeutics and therapeutic targets to control brain injury and to the characterization of biomarkers for safer staging of the disease.

Funding

The Wellcome Trust (grant codes: 073542; 080268).

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doi:10.1016/j.drudis.2010.09.388