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Cell penetrating peptides to dissect host-pathogen protein-protein interactions in *Theileria*-transformed leukocytes

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ABSTRACT

One powerful application of cell penetrating peptides is the delivery into cells of molecules that function as specific competitors or inhibitors of protein-protein interactions. Ablating defined protein-protein interactions is a refined way to explore their contribution to a particular cellular phenotype in a given disease context. Cell-penetrating peptides can be synthetically constrained through various chemical modifications that stabilize a given structural fold with the potential to improve competitive binding to specific targets. *Theileria*-transformed leukocytes display high PKA activity, but PKA is an enzyme that plays key roles in multiple cellular processes; consequently genetic ablation of kinase activity gives rise to a myriad of confounding phenotypes. By contrast, ablation of a specific kinase-substrate interaction has the potential to give more refined information and we illustrate this here by describing how surgically ablating PKA interactions with BAD gives precise information on the type of glycolysis performed by *Theileria*-transformed leukocytes. In addition, we provide two other examples of how ablating specific protein-protein interactions in *Theileria*-infected leukocytes leads to precise phenotypes and argue that constrained penetrating peptides have great therapeutic potential to combat infectious diseases in general.

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1. Introduction

1.1. General Introduction to bovine leukocytes infected and transformed by Theileria parasites

Theileria is an intracellular protozoan transmitted by ticks, which affects ruminants. Tropical theileriosis exhibits many features of human leukemia and is caused by *Theileria annulata*, whereas *Theileria parva* causes a lymphoma-like disease called East Coast Fever. *T. annulata* infects bovine B cells and macrophages/monocytes, while *T. parva* infects bovine B and T lymphocytes.[7,18] When infected, the leukocytes become fully transformed and disseminate throughout the animal infiltrating both lymphoid and non-lymphoid tissues (lungs and gastrointestinal tract). Moreover, when *Theileria*-transformed macrophages are injected into immune-compromised mice they form disseminating tumors.[20,46,62] Another cancer-like phenotype is that *in vitro Theileria*-transformed leukocytes proliferate in an uncontrolled manner and do not require exogenous growth factors.[18] Importantly, *Theileria*-dependent transformation is entirely reversible upon theilericidal drug treatment and so does not involve irreversible changes (mutations) to the host genome. The theilericidal drug buparvaquone specifically kills the parasite and the previously transformed leukocytes stop proliferating and regain their dependence on exogenous growth factors.[19]

Importantly, live attenuated vaccines exist to tropical theileriosis[15] that are generated by multiple passages of infected macrophages, which with time become attenuated for virulence i.e. they lose their hyper-disseminating virulence trait.[2,20,32] A number of studies have provided evidence on how infection manipulates leukocyte signal transduction pathways, contributing to different aspects cellular transformation.[7,8,17,18] Thus, *Theileria*-provoked leukocyte transformation provides a unique, reversible model with the potential to provide key insights relevant to leukemia. Here, we discuss the use of penetrating peptides to competitively ablate specific protein-protein interactions in infected cells and the consequences this has on tumor phenotypes of *Theileria*-transformed leukocytes.

1.2. Cell penetrating peptides (CPPs)

CPPs have gained much attention due to their ability to enter cells via a receptor-independent mechanism and deliver biologically active cargo molecules into the cell interior.[38] The protein transduction domain (PTD) of the CPPs is made up of 3–30 amino acids and possesses an overall positive charge, but has no specific consensus sequence.[16] Many CPPs have been successfully given to different mammalian cell populations to deliver different types of 'cargo' molecules, including peptides, oligonucleotides, proteins, and drug-loaded nanoparticles.[36] Moreover, CPPs have been employed to deliver a variety of therapeutics to different types of cancer cells,[58,64] including breast cancer,[43] carcinoma[28] and melanoma.[35] One powerful application of CPPs is the delivery of molecules that function as specific competitors or blockers of protein-protein interactions into the intracellular environment. Ablating defined protein-protein interactions is a refined way to explore their contribution to a particular cellular phenotype in a given disease context.

Many studies have shown the therapeutic application of CPPs for the treatment of a variety of hyper-proliferating cells.[39,14] For example, a CPP-based chimeric protein bound to the CrkL-SH3N interface caused a significant decrease in the formation of p210Bcr-Abl-CrkL complexes in Chronic Myeloid Leukemia.[39] Furthermore, it also caused a significant decrease in the *in vitro* proliferation index of primary CML cells and cell lines derived from BCR-ABL-positive patients.[40] CPP-mediated disruption of SH3 domain-dependent complexes, as a strategy for inhibition of specific signaling pathways has been tested in several studies[14] {Posern, 1998 #55[55]}.

Unconstrained peptides may have numerous shortcomings including susceptibility to proteolysis, loss of secondary structural fold and inability to permeate cell membranes. By synthetically constraining a peptide sequence through various chemical modifications that stabilize a structural fold such as an alpha-helix, beta-turn or loop structure, the peptide may have entropically favorable properties for binding.[42] Further, constrained peptides can offer many additional attributes including retention of secondary structural folds, cell permeability, improved target affinity and resistance to proteolytic degradation.[65,67,34] Given these properties, constrained peptides have been used to disrupt a variety of protein-protein interactions.[33,68,56]

2. Results and discussion

2.1. Inhibition of Theileria-induced leukocyte transformation by cell-penetrating peptides: Grb2 penetrating SH3 domain competing peptide

Grb2 (Growth factor receptor-bound protein 2) serves as a potentially interesting adaptor to target in the context of host-pathogen interactions and was identified from examination of the Ode vaccine line against tropical theileriosis. Previous microarray analyses indicated a significant decrease in macrophage transcription of *Grb2* in the live attenuated vaccine line, as opposed to its virulent transformed progenitor macrophage cell line.[5] Grb2 is an adaptor protein that participates in signal transduction pathways.[66] Grb2 contains three domains; one Src Homology 2 (SH2) and two Src Homology 3 (SH3) domains located at the N- and C-termini.[48,4] The two SH3 domains form a direct complex with the proline-rich regions of the other partner proteins, while the SH2 domain binds to tyrosine phosphorylated peptides in specific receptors.[73]

We have previously shown that *Theileria*-infected macrophages produce many pro-inflammatory cytokines such as transforming