Working with Cell-Penetrating Peptide (CPP) Trans-Activating Tranductions (TATs): Risk Assessment and Biosafety Recommendation

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Introduction

• Cell-Penetrating Peptide (CPP)
  – Able to translocate across the plasma membrane of eukaryotic cell
  – Use for intracellular delivery

• Trans-Activating Tranductions (TATs):
  – Potent trans-activator of HIV-1
  – Essential for viral replication
  – Able to trans-activate of other cellular genes
  – Can leave cells from which is synthesized and cross the membrane of adjacent cell, where it localizes in the nucleus
• Amino acid sequence of HIV-1 TAT

• Potential hazardous of TAT protein:
  – Immunosuppressive -> induce apoptosis CD4+ T cell, induce abnormalities in macrophage and APC
  – Cytotoxic activity -> expression of HIV-1 TAT on brain can cause a number of developmental and behavioral abnormalities
  – Carcinogenic property -> stimulation of cell growth and has potent angiogenic activity
Risk Assessment

- TAT transduction domains only:
  - not infectious
  - not replicate
  - not transfer any genetical material
  - Sero-conversion due to TAT-residues are very scarce

- Nature of the proteins being fused to TAT possibly:
  - Immunogenic
  - Toxins
  - Oncogen

- Fusion proteins potential:
  - Enter through skin
  - Induce MHC-I
Biosafety Recommendation (1)

• Biosafety Level 1:
  – Cloning of sequences coding for TAT-fusion proteins in \textit{E. coli}
  – Plasmid preparation
Biosafety Recommendation (2)

• Biosafety Level 2: Expression of TAT-fusion protein
  – Proteins must be handled as hazardous material
    • Labcoat with front close, mouth pipetting is not allowed, masker, disposable gloves, safety google, avoid direct contact with the skin, cuts, or mucous membrane
    • Work in BSC or plastic backed absorbent lab paper in bench
Biosafety Recommendation (3)

• Decontamination Procedure
  – Wear PPE (labcoat, gloves, goggle, masker)
  – Decontaminate work surface using detergent with a protease enzyme for minimum 20 minutes
Disposal Procedures

• Deactivate and dispose of TAT solutions and cultures using standard autoclave methods

• Deactivate solution using a 1:10 dilution of bleach (sodium hypochlorite) in a 1:1 mixture with TAT solution
  – Dispose of solution down the sewer drain with copious amounts of water
Wash hand after working with TAT material
References


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Abstract
Cell-penetrating peptides (CPPs) are peptides which able to cross cellular membrane and able to be a non-invasive vector since it can bring other molecules such as small RNA/ DNA, plasmids, antibodies, and nanoparticles to be transported into cells as its cargo. Trans-activating transduction (TAT) proteins are 14 kDa proteins transcribed from complex spliced mRNAs which function intracellularly as a trans-activating factor of the human immunodeficiency virus type 1 (HIV-1). Some studies showed that exposure to Tat protein can lead to a serious health consequences even in the absence of HIV infection because it has potential immunosuppressive, cytotoxic, and carcinogenic properties.

Keywords:
Cell-penetrating peptides (CPP), Trans-activating transduction (TAT) protein, Human immunodeficiency virus type 1 (HIV-1), health consequences

References