

Interaction of transduction enhancing peptide nanofibrils with cells and virions assessed by complementary EM techniques

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Background and aims:

Retroviral gene therapies are applied to treat cancers. In chimeric antigen receptor (CAR) T-cell therapy the gene for the fusion protein CAR is inserted into the patients' T-cell genome by retroviral transduction *in vitro*. After reintroduction into the patient, the reprogrammed T-cells expressing CAR are able to recognize and destroy cancer cells (Cappell and Kochenderfer, 2023). For cost efficient application of CAR T-cell therapy, researchers aim at enhancing transduction efficiencies. Transduction efficiencies are often low due to the low retroviral titers. Another reason is the electrostatic repulsion between the negatively charged virions and the negatively charged plasma membrane. To overcome this repulsion, positively charged peptide nanofibrils (PNF) were developed. One type of PNF, enhancing factor C (EF-C), is actively engaged by cellular protrusions and leads to a 9.3-fold enhanced transduction rate in HeLa cells (Schütz et al., 2021). However, EF-C fibrils formed large aggregates, which could clog the bloodstream when introduced into the patient and are, thus, not suitable for clinical application. A novel peptide, Derivative-4 (D4), was identified by *in-silico* screening (Rauch-Wirth et al., 2023). To better understand the morphology of D4 PNF and to characterize their interaction with virions and cells, electron microscopy was performed.

Methods:

The morphology of D4 PNF was characterized by negative staining and transmission electron microscopy (TEM). The binding of D4 to virions and the cell surface was then studied by scanning electron microscopy (SEM) of cells after critical point drying. To obtain a more complete view of binding of D4 to cells, samples were analyzed by TEM and STEM tomography. For both, high-pressure frozen and freeze-substituted cells were used (Bergner et al., 2022).

Results:

TEM showed that D4 PNF are shorter than EF-C. In cell culture, most of the D4 aggregates were formed by structures measuring up to a few hundred nanometers in length ("D4 network"). SEM analysis revealed that D4 networks bind to cells via cellular protrusions. When the networks were considerably smaller than the cell, the plasma membrane formed indentations close to the network, indicated starting cellular uptake. TEM analysis of cell cultures treated with PNF and virions revealed a second D4 morphology; besides D4 networks also aggregates of μm -long, thick D4 nanofibrils were found. We then analyzed virion binding patterns to these different D4 morphologies by TEM and STEM tomography. Irrespective of the D4 morphology, virions bound to D4 aggregates. However, the D4 morphology appeared to influence virion binding. Virions bound to the surface of the D4 networks and were also found within the network. In contrast, at aggregates formed by the long and thick D4 fibrils, virions were only found on the surface of the aggregate.

Conclusion:

Using 4 complementary EM techniques, we obtained a better understanding of the transduction enhancing function of D4. This understanding is important for the further development of D4 PNF and their clinical applications.

Keywords:

STEM tomography, SEM, gene therapy

Reference:

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