# Introducing Cytonacx, RNTein Biotech Lab. latest biotechnology in the field of molecular therapy

RNTein Biotech. Lab. announces the generation of Cytonacx. With its improved size of 80-200 nm, this nanoparticle shows high transfection efficiency and high cell and tissue specificity, without involving viral genomes. Cytonacx is an enveloped therapeutic bionanoparticle that will be able to bind to cell-surface receptors and engage in target cell membrane fusion. Moreover, as it can be assembled in vitro, Cytonacx is feasible to mass-produce.

With PEGylation, Cytonacx exhibits its natural properties of risk-free and long-lasting gene transduction, overcoming limitations of viral vectors and synthetic liposomes in gene therapy. Cytonacx is created from a particular type of bionanoparticles able to cure human diseases such as cancer or AIDS.

## I: Cancer suppression by Cytonacx/*CDC6* shRNA.

Research results have shown that tumors possess a unique physiology of fenestrated vasculature and poor lymphatic drainage, a characteristic that is now widely known as the enhanced permeability and retention (EPR) effect. Relatively larger gaps between adjacent endothelial cells in tumor neovasculature allows for passive targeting to the tumor site, while poor lymphatic drainage leads to relatively high retention of macromolecular therapeutics within the tumor mass (Matsumura and Maeda, 1986).

Over the past few decades significant advances have been made in fundamental cancer biology, allowing for remarkable advances in the diagnosis and therapy of cancer. However, the clinical translation of these advances lags far behind. A major hurdle is the successful delivery of novel therapeutic agents to the target site, while avoiding adverse damage resulting from systemic administration.

Cytonacx carrying *CDC6* shRNA-producing plasmid DNA would be a promising candidate for molecular therapy of cancer. Human Cdc6, a homologous of highly conserved yeast DNA replicative protein, is essential for the initiation of DNA replication. Cdc6 depletion by using *CDC6* siRNAs has shown to induce apoptosis in human hepatocarcinoma cell lines and cervical carcinoma HeLa cells. We have generated a biotech system by producing *CDC6* short hairpin RNA (shRNA), which can be converted to *CDC6* siRNA in human cancer cells. We have shown that Cdc6 knockdown *via* infection of human neuroblastoma cells with *CDC6* shRNA-producing lentivirus induces the programmed cell death (Feng et al., 2008). Moreover, infection of human breast carcinoma cells with the virus induces proliferative senescence (Feng et al., 2008, manuscript).

In human cells, the levels of Cdc6 change markedly in response to various stresses. High level of Cdc6 has been found to promote the heterochromatinization of the p14 regulatory domain (RDp14), and has been

associated with the oncogenic activities in human cancers (Gonzalez, et al., 2006). The inhibition of endogenous Cdc6 *via* Cdc6 knockdown results in an increased G1 content with decreased S fraction in cells with p53 deficiency (Duursma and Agami, 2005).

Mutations of p53 gene has been found in about 50% of human tumors, and the tumor cells are defective in DNA damage checkpoints with compromised cell-cycle arrest and decreased apoptosis (Bartkova et al., 2005). Cdc6 could be more stable in tumor cells, due to p53 deficiency and due to strong Cdc6 phosphorylation by activated cyclin E-cdk2 kinase (Mailand and Diffley, 2005). Therefore, Cdc6 may act as a powerful engine to drive tumor cell growth, while the cyclin E-cdk2 kinase fuels this unlimited cell proliferation. As Cdc6 knockdown causes the loss of proliferative capability in those tumor cells with p53 deficiency, it is interesting to determine whether the suppression of Cdc6 *via* Cytonacx/*CDC6* shRNA would stop the unlimited proliferation of human cancer cells, therefore benefits us in cancer treatment.

## II. Blocking HIV-1 infection with Cytonacx/FEN 1-D181A.

Human immunodeficiency virus type 1 (HIV-1) infects $CD4^+$ cells and causes progressive depletion of lymphocytes. During infection, HIV-1 central DNA flap is formed in the process of reverse transcription of HIV-1 RNA. The central DNA flap has been shown to be a structural determinant in facilitating nuclear import of the HIV-1 PIC in both dividing and nondividing cells (Zennou et al., 2000). The central DNA flap must be cleaved before HIV-1 complete virus replication. However, no viral protein which can cleave the overlap or repair internal nicks or gaps has been identified so far.

The HIV-1 central DNA flap possesses all of the substrate characteristics required for enzyme cleavage by a cellular flap endonuclease 1 (FEN-1). Recombinant hFEN-1 cleaves an overlap oligonucleotide in an HIV-1 flap model substrate (Rumbaugh et al., 1998), and is capable of cleaving 5' overhangs in substrates derived from the HIV-1 cDNA ends when HIV-1 RT and DNA ligase are present in an in vitro assay (Brin et al., 2000). These results indicate that hFEN-1 likely plays a role in cleaving the HIV-1 central DNA flap in vivo.

A deficiency in hFEN-1 function, a single amino-acid residue of the $181^{st}$ Asp changed to Ala (D181A form of FEN-1) has been identified during the investigation of HIV-1 infection (Feng et al., 2004 manuscript). D181A is a dominant negative mutant which is deficient in endonuclease cleavage. This FEN-1 mutant fails to cut off the central DNA flap. The failure of cleaving HIV-1 central DNA flap causes an impaired HIV-1 integration, inhibiting HIV-1 replication efficiently. Though the mutant hFEN-1 has shown the inhibition of the wild-type hFEN-1 endonuclease activity in vitro (Shen et al., 1997), little abnormality regarding cell growth has been observed upon the expression of the

D181A protein, while HIV-1 reverse transcriptase activities sharply low in the HIV-1 virus-infected cells (Feng et al., 2004, manuscript). It is of importance that the D181A protein renders the HIV-1 permissive human lymphocytes to be resistant to the infection without creating detectable harm to the cell.

It is known that current anti-AIDS studies focus on the discovery of HIV-1 viral proteins inhibitors. However, as these viral proteins critical for integration are virus-encoded, they are subject to a high rate of mutation, which can lead to drug resistance. This rapid development of resistance should not occur with drugs that can target cellular functions necessary to complete the integration process. The mutated hFEN-1 protein is found in association with the HIV-1 reverse transcriptase (RT), which enables us to target the HIV-1 viral protein *via* Cytonacx producing the D181A form of FEN-1. Cytonacx/FEN-1-D181A is such a therapeutic model. To target HIV-1 specifically, the envelop protein in the Cytonacx/FEN-1-D181A will be modified recombinant HIV-1 gp120 which recognizes but not harms human $CD4^+$ cells. Hematopoietic stem cells can also be transduced with Cytonacx/FEN-1-D181A. This approach is necessary because stem cells engineered for producing D181A form FEN-1 can proliferate and create long-lasting anti-HIV therapy.

## References

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