DREAM

Drug delivery nanosystem for HPV infection therapy Nanotechnologies

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TEAM

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HPRD Health Products Research and Development Lda
Association of Instituto Superior Técnico for Research and Development (IST-ID)
Center for Nuclear Sciences and Technologies (C2TN)
Hospital Center of Cova da Beira (CHUCB)

NANOTECHNOLOGIES

Background

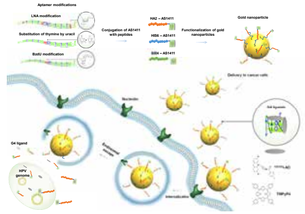
Human papillomaviruses (HPVs) are largely accepted as major etiologic agents for cervical cancer. The implementation of screening and vaccination programs for HPV have reduced the cancer incidence and mortality. Although there still a need to treat patients infected and in precancerous state, able to clear HPV infection in the early stages of infection.
Nanotechnology has emerged as a potential therapeutic approach to viral infection and cancer treatment, specifically, DNA-based nanoparticles for antiviral and/or anticancer drugs greatly enhance their bioavailability and increase their target specificity.
AS1411 is a G-quadruplex (G4)-forming DNA oligonucleotide that functions as an aptamer of nucleolin (NCL), a protein overexpressed in cancer cells. Two preliminary studies showed the involvement of NCL in expression of HPV18 oncogene after oligonucleotide binding and HPV16 genome stability. AS1411 has been used as a drug delivery system and have cellular internalization, and anticancer activity by interfering with NCL oncogenic functions. Clinical trials of AS1411 have indicated that it is well tolerated with evidence of therapeutic activity, but improved pharmacology and potency may be required for optimal efficacy. Since G-rich regions have been found in HPVs (LCR, E1, E4 regions) such potential anticancer/antiviral ligands may be a promising new therapeutic strategy against HPV infection.

Approach/Methodology

AS1411 derivatives with chemical modifications (LNA and uracil) and number/position of the bases (AT11, AT11-L0 and AT11-B0) were introduced to improve the binding affinity to NCL and pharmacological properties. The association of G4 ligands to AS1411 derivatives was evaluated by biophysical techniques (NMR, circular dichroism, Fret-melting, fluorometric titrations). Additional G quadruplex sequences were evaluated by the Ellington lab, using a novel chip-based approach. Some ligands have a dual effect: G4 maintenance/stabilization and antiviral /anticancer activity towards cervical cancer cell lines and organotypic cultures infected with HPVs 16 and 18. AS1411 derivatives attached to G4 ligands gold nanoparticles and micelles (vehicle-ligand nanosystems) increase accumulation in cancer cells.
The antitumor efficacy of the vehicles-ligands was evaluated in cancer cells, fibroblasts and biopsies from cervical cancer patients of CHUCB hospital. Antiviral activity was assessed in collaboration with Prof. Craig Meyers from the USA. Organotypic cultures were infected with HPVs 16 and 18 and the viral titer, encapsidation and number of virions determined. Labfit-HPRD used the most promising vehicle-ligand to do permeation studies in porcine cervical tissues, based on similar tissue responses between human and porcine vagina. The formulation of a topical delivery system to control HPV replication was performed to target infected and basal cells.

Implementation Challenges

The main challenges were to implement reproducible gold nanoparticles conjugated with the DNA and to efficiently internalize these nanoparticles in the topical formulation. Moreover, established stable organotypic cultures with episomal high risk HPVs since all the laboratories of the partners involved in DREAM have only BSL2 security. To do it the Ellington lab has identified a cell-penetrating aptamer, and C. Cruz established a new collaboration with Prof. Craig Meyers from USA who have this expertise and BSL3 laboratories.



Main Findings

The production of AS1411 derivatives with chemical modifications (LNA and uracil derivatives) was not able to improve the NCL affinity as well as their pharmacological properties. However, derivatives AT11, AT11-L0 and AT11-B0 showed improvement in selectivity, affinity, and in vitro target to NCL.
The addition of AS1411 to gold nanoparticles and micelles improved the selectivity to cancer cells. Conjugation to nanoparticles led to a decrease of the time required for the AS1411 perform its biological effect in the NCL-positive cells and nanoparticles were not captured by the lysosome. The presence of AS1411 on the micelle surface promoted the specific accumulation of the G4 ligands in cervical cancer cells which inhibited their growth, with little effect on non-malignant normal cells.
The micelles were able to penetrate cervix tissue biopsies of patients with HPV infection in precancerous stages, demonstrating the potential to treat HPV infection in its latent stage, preventing oncogenesis and cervical cancer progression.
The biopsies provided by the CHUCB expressed high levels of NCL.
In collaboration with HPRD company, selected nanoparticles were incorporated in a topical formulation and applied in porcine cervical tissues to determine permeation. The nanoparticles permeated and were retained in the tissue.

Expected Impact

The development of improved AS1411 derivatives, and association with nanoparticles augments available technological approaches to cancer treatment, as does the development of new screens for quadruplexes and the development of cell-penetrating aptamers. The improvement of the ligands selectivity to HPV infected cells obtained through the conjugation with the AS1411-gold nanoparticles demonstrates the ability of these systems to improve the selectivity of potential anticancer/antivirals that in normal situations cannot be applied due to their toxicity in healthy tissues, but through their conjugation with the nanoparticles was able to overtake this roadblock. This is an important finding that can be implemented in the pharmaceutical industry because the preliminary ex vivo permeation studies demonstrated the efficacy of the formulation in penetrating and accumulating the nanosystems in the target cells.
The micelles were also able to penetrate cervix tissue biopsies of patients with HPV infection in precancerous stages, demonstrating the potential of this system to treat HPV infection in its latent stage, preventing oncogenesis and cervical cancer progression. The administration route of the drug-loaded nanosystems was also addressed. The C8-loaded micelles were formulated as a gel for local application in the female genital tract, specifically tissue with precancerous lesions and/or HPV infection.

Project Highlights

- The biopsies of patients with HPV infection (low and high grade) provided by the CHUCB hospital and IPATIMUP expressed high levels of NCL.
- Ethics Committee approval from CHUCB study number 92/2018 to manipulate biological material from women infected with HPV.
- Establishment of stable organotypic cultures with episomal high risk HPVs 16 and 18 providing new collaborations.
- Development of drug-loaded AS1411-functionalized micelles, and their local administration in vaginal tissue using gel formulations.
- Conjugation of AS1411 with the gold nanoparticles improved the selectivity to the cervical cancer cells.

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