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Biophysical Targeting of OmpF Nano-Channel Forming Proteins, Translocating Ofloxacin Antibiotic into E.Coli, Involved In Urinary Tract Infection

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OmpF is an ion channel in outer membrane of E.coli that is responsible for translocation of molecules with an exclusion limit of 600Da. These channels are known for the path they form for translocation of hydrophilic molecules, antibiotics, needed to pass through the hydrophobic membrane. An efficient antibiotic is expected to exhibit high affinity for certain target cell and molecules and reach them rapidly. Quinolons and Beta-lactames are among the successful and widely used antibiotics known to cure bacterial infections at present. The antibiotic that enter the bacteria kill them by inhibiting DNA gyrase or the topoisomerase type 2 enzymes as a target, thereby interfering the DNA replication and translocationIn this work, we investigated the passage of Ofloxacin antibiotic through the single OmpF porin of E.coli by means of voltage clamp technique in real time to address the drug trafficking in urinary tract infection disease in human. We also investigated the effect of the passing antibiotic on channel gating, conductance, and voltage sensitivity. The experimental work was further elaborated by theoretical and modeling approaches to address the exact electrostatic effect of antibiotic on the lining group in particular those at eyelet area of the nano-channel that constrict it to a diameter of about 0.4nm. Our results showed that the Ofloxacin is not soluble in lipid phase and did not pass through the membrane. Although the voltage sensitivity of the channel was not changed in the presence of the antibiotics, the frequency of gating increased and fast flickering was caused due to the transient obstruction. The conductance of the channel also remained the same indicating lack of any binding and conformation induction caused by the antibiotic.

Keywords: OmpF Channel, Ofloxacin Antibiotic, Urinary Tract Infection, Translocation.

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Targeting Mesothelin for Treatment of Cancer

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Mesothelin, a differentiation antigen present in a series ofmalignancies such as mesothelioma, ovarian, lung and pancreaticcancer, has been studied as a marker for diagnosis and a target forimmunotherapy. We, however, were interested in evaluating theeffects of direct targeting of Mesothelin on the viability of cancercells as the first step towards developing a novel therapeuticstrategy. We report here that gene specific silencing for Mesothelinby distinct methods (siRNA and miRNA) decreased viability ofcancer cells from different origins such as mesothelioma (H2373),ovarian cancer (Skov3 and Ovcar-5) and pancreatic cancer(Miapaca2 and Panc-1). Additionally, the invasiveness of cancercells was also significantly decreased upon such treatment. We theninvestigated pro-oncogenic signaling characteristics of cells uponmesothelin-silencing which revealed a significant decrease inphospho-ERK1 and PI3K/AKT activity. The molecular mechanism ofreduced invasiveness was connected to the reduced expression ofβ-Catenin, an important marker of EMT (epithelial-mesenchymaltransition). Ero1, a protein involved in clearing unfolded proteins anda member of the ER-Stress (endoplasmic reticulum-stress) pathwaywas also markedly reduced. Furthermore, Mesothelin silencingcaused a significant increase in fraction of cancer cells in S-phase.In next step, treatment of ovarian cancer cells (OVca429) with alentivirus expressing anti-mesothelin microRNA resulted insignificant loss of viability, invasiveness, and morphologicalalterations. Therefore, we propose the inhibition of Mesothelin as apotential novel strategy for targeting human malignancies.

Keywords: Mesothelin, Cancer, Immunotherapy, Pro-oncogenic Signaling.