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COMPARATIVE ANALYSIS OF QUERCETIN AND TAXIFOLIN INTERACTION WITH HUMAN TELOMERIC G-QUADRUPLEX DNA HYBRID FORM BASED ON MOLECULAR DYNAMIC SIMULATIONS

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G-quadruplex stabilizing ligands demonstrated their capability to decrease cell growth *in vitro* and *in vivo* cancer models. After more than a decade of investigations aimed at targeting telomeric G-quadruplexes, the development of G-quadruplex-based binding ligands has progressed slowly with only one candidate making it to clinical trials. The most urgent problems are the lack of high-resolution structural data for many G-quadruplex forms and small molecules' low selectivity.

Two 400 ns molecular dynamic simulations of quercetin and taxifolin interactions with hybrid form of human telomeric G-quadruplex DNA were conducted. Binding modes of ligands were identified. Energetic parameters and hydrogen bonds formations were calculated. The interaction of taxifolin with G-quadruplex loop is an obvious example how slight difference between chemical compounds could define binding mode.

G-quadruplex – telomeres – molecular dynamic simulation – cancer

In vitro և *in vivo* քաղցկեղային մոդելներում Գ-քվադրուպլեքսներ կայունացնող լիգանդները ցուցաբերում են բջիջների աճի նվազեցման ունակություն: Ուսումնասիրությունները, կապված Գ-քվադրուպլեքսներ կապող լիգանդների հետ, դանդաղ ընթացք են ունեցել, և միայն մեկ միացություն է հասել կլինիկական հետազոտությունների փուլի: Դրա հիմնական պատճառներն են բազմաթիվ Գ-քվադրուպլեքսների բարձր ճշգրտրտության կառուցվածքային ձևերի վերաբերյալ տվյալների տեղեկատվության բացակայությունը և լիգանդների ցածր ընտրողականու-թյունը:

Իրականացրել են քվերցետինի և տաքսիֆոլինի փոխազդեցության երկու 400 նվ մոլեկու-լային դինամիկ սիմուլյացիաներ մարդու Գ-քվադրուպլեքսային թելոմերներային ԴՆԹ-ի հետ: Պար-զաբանվելեն լիգանդների կապման ձևերը: Հաշվարկվել են էներգետիկ պարամետրեր և ջրածնային կապի ձևավորումները: Տաքսիֆոլինի փոխազդեցությունը Գ-քվադրուպլեքսի հանգույցի հետ լավ օրինակ է, թե ինչպես քիմիական միացությունների փոքր տարբերությունը կարող է սահմանել լիգանդի կապման ձևը:

Գ-քվադրուպլեքս – թելոմերներ – մոլեկուլային դինամիկ սիմուլյացիա – քաղցկեղ

G-квадруплекс стабилизирующие лиганды проявляют цитотоксичность в отношении роста раковых клеток в *in vivo* и *in vitro* моделях. В результате десятилетних исследований взаимодействия низкомолекулярных лигандов с G-квадруплексными структурами было вы-явлено всего одно соединение, достигшее клинических испытаний. Основными причинами этого являются отсутствие структурных данных высокого разрешения множества G-квадру-плексных форм, а также низкая селективность исследуемых лигандов.

Были проведены две 400 нс молекулярно динамические симуляции взаимодействия кверцетина и таксифолина с теломерной G-квадруплексной ДНК человека. Были выявлены сайты связывания лигандов. Были рассчитаны энергетические параметры и водородные связи. Взаимодействие таксифолина с петлей G-квадруплекса наглядно демонстрирует роль незначительных различий в химической структуре лигандов в их моде связывания.

G-квадруплекс – теломеры – молекулярно динамическая симуляция – рак

Telomere is a region of repetitive nucleotide sequences at end of a chromosomes which prevents them from non-homologous pairing, damage during cell division, exonuclease and ligase activities [1]. The telomeric ends of human DNA consist of tandem repeats of $d(TTAGGG)_n$ 5-10 kb hexanucleotide sequences that form a terminal single-chain 3'-overhead [2]. The structure and stability of telomeres is related to cancer, aging, genome maintaining and genetic stability [3]. The G-quadruplex structures that form at the distal 3' end of the human telomeres prevent hybridization of the DNA single strand with the telomerase RNA template and therefore was considered as a potential target for new anticancer therapy based on the use of low molecular weight ligands [4]. G-quadruplex stabilizing ligands demonstrated their capability to decrease cell growth *in vitro* and *in vivo* cancer models [5, 6].

Based on the molecular structure and functions, dihydroquercetin (taxifolin) is close to quercetin and routine, but it surpasses them by pharmacobiological activity: it is not mutagenic and less toxic than the mentioned compounds [7]. Taxifolin is known for its anti-proliferative effects on different types of cancer cells by inhibiting cancer cell lipogenesis [8].

After more than a decade of investigations aimed to targeting telomeric G-quadruplexes, the development of G-quadruplex-based binding ligands has progressed slowly with only one candidate making it to clinical trials [9]. One of the reasons is the lack of high-resolution structural data for many G-quadruplex forms and G-quadruplex binding ligands low selectivity. Another problem is the dynamic polymorphism of these structures and the limitations of traditional biophysical methods in the study of this phenomenon [10]. Low-resolution spectroscopic methods, such as circular dichroism or UV spectroscopy, are not accurate enough to determine the differences between structural conformers within ensemble. High resolution methods, such as Nuclear Magnetic Resonance (NMR) and crystallography also provide limited information in the study of structural polymorphism of G-DNA structures [11].

Molecular modeling and simulations are powerful tools to provide detailed structural and dynamic information to decipher the binding nature of DNA ligands [12].
In this study, we conducted molecular dynamics simulations with a free ligand to decipher the binding modes and interaction features of quercetin and taxifolin to intramolecular human telomeric G-quadruplex structure. Major binding poses were identified and detailed binding pathways were characterized.

Materials and methods. Intermolecular telomeric G-DNA structure were obtained from Protein Data Bank (PDB ID: 2HY9) [13]. It's a hybrid G-DNA topology formed in K^+ solution. This topology also has AAA triplet that naturally occurs, capping the top tetrad of the hybrid-type telomeric G-quadruplex. This adenine triple was reported to play an important role in the formation and stabilization of human telomeric G-quadruplex structure in K^+ solution [14]. The first model of NMR ensemble was used as the best representative conformer. 2D model of quercetin and taxifolin were obtained from PubChem [15]. Quercetin and taxifolin 3D parameters for molecular dynamic simulations were generated using the acpype tool [16] for the General Amber Force Field [17] with AM1-BCC partial charges. ACPYPE (AnteChamber PYthon Parser interfacE) is a python script within the ANTECHAMBER software that generates small molecule topologies and parameters for GROMACS and other MD software. Figures of ligands interaction

with G-quadruplex DNA structure were obtained with using of VMD tool [18]. Diagrams and plots of electrostatic and Van der Waals (VdW) energies were obtained using xmgrace module of GROMACS software. Hydrogen bond identification diagram was obtained using matplotlib [19].

We conducted two Molecular Dynamics (MD) simulations of G-DNA with taxifolin and quercetin using the GROMACS suite, v 2016.5 [20]. Amber ff99SB-ILDN force field was used for the MD simulations [21]. In all cases, Short-range non-bonded interactions were cut off at 1.4 nm. Particle Mesh Ewald (PME) was used for the calculation of long-range electrostatics. A time step of 2 fs was used during heating, the production run, and coordinates were recorded every 2 ps. Each MD simulation of 200 ns were performed.

Structures were placed in a dodecahedron box of TIP3P water, to which additional K^+ counter-ions were added for neutralizing system. After that two steepest descents minimization were performed and then equilibrated in two stages. The first stage involved simulating for 200 ps under a constant volume (NVT) ensemble. The second stage involved simulating for 2000 ps under a constant-pressure (NPT) for maintaining pressure isotropically at 1.0 bar [22]. The temperature was sustained at 300 K using V-rescale algorithm. For isotropic regulation of the pressure, the Parrinello-Rahman barostat was used.

Mdtraj were used for identification of hydrogen bonds formation between quercetin and taxifolin with human telomeric G-quadruplex. Gromacs energy module was used for the calculations of VdW and electrostatic energy and rms module was used for RMSD calculations.

Results and Discussion. *Telomeric G-DNA and taxifolin ab initio simulation.* Starting from unbound state (fig. 1A) taxifolin finds its binding mode within first 55 ns of simulation. Taxifolin interacts with the top of DNA molecule inducing conformational changes to the AAA loop, especially to adenin26 and adenin15 (fig. 1B). Both molecules are stable during 200 ns simulation (fig. 2E). Based on VdW (fig. 1F) and electrostatic energy calculations (fig. 1G) and hydrogen bond identification analysis (fig. 1H) it's more likely that interaction between taxifolin and hybrid form of telomeric human G-quadruplex DNA is mostly driven by electrostatic forces. To confirm taxifolin binding mode, the simulation was extended to 400 ns, during which taxifolin maintained its binding state.

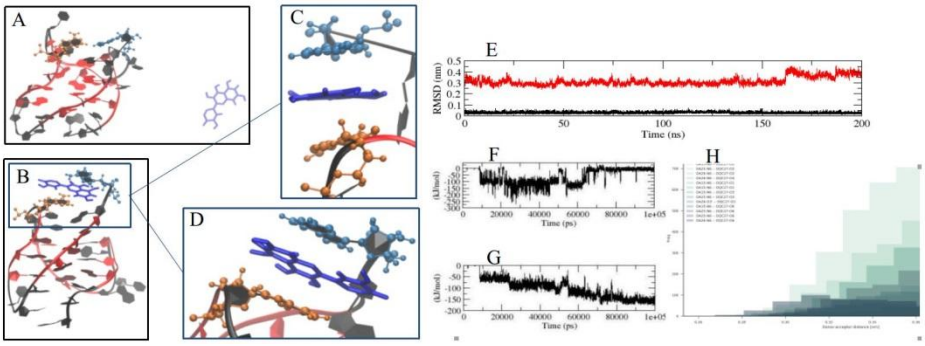

Fig. 1. A) Starting point of simulation; taxifolin (left right corner) is in unbound state. B) Interaction of taxifolin with adenin26 and adenin15. C and D) zoomed view of taxifolin binding mode with hybrid human telomeric G-quadruplex DNA. F) Electrostatic energies. G) VdW energies. H) Mdtraj hydrogen identification results. 1 weak hydrogen bond forms in 200 ns.

Telomeric G-DNA and quercetin ab initio simulation. Starting from unbound state (fig. 2A) quercetin interacts with G-DNA by stacking to the top of molecule and inducing conformational changes to the loop at the bottom side of molecule similar as in taxifolin-G-DNA simulation, but on the opposite side of molecule (fig 2B). Then it quickly loses its binding state and drops out. It starting to move towards to G-DNA groove until it reaches its final binding site (fig. 2C). Quercetin finds its binding mode in about 80 ns. Starting from 60 ns quercetin intercalating to G-DNA in the groove and after stabilizing, maintains its conformation till the end of the 200 ns simulation. At the beginning both

molecules endure low structural fluctuations and stabilize after ligand reaches its final binding state (fig. 2G). According to electrostatic and VdW energies calculation and hydrogen bonds identification results (fig. 2E, F, H respectively) quercetin intercalation is accompanied by high electrostatic forces and stabilized by at least two strong hydrogen bonds. To confirm quercetin binding mode, simulation was also extended to 400 ns, during which quercetin maintained its intercalation binding mode.

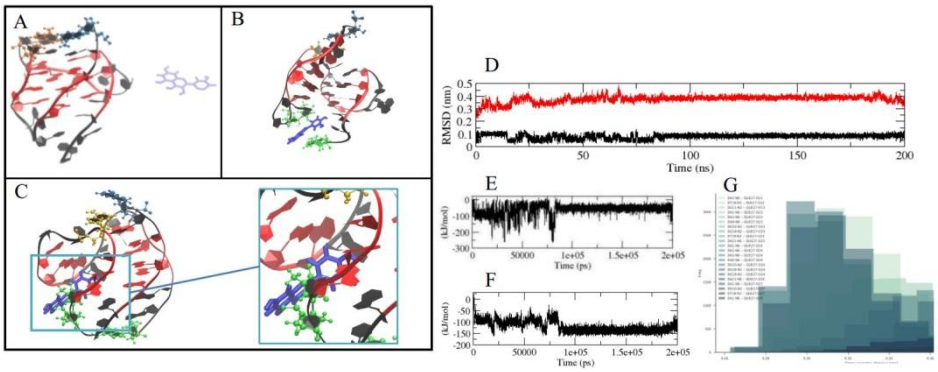

Fig. 2. A) Starting point of simulation. Quercetin is in unbound state. B) Quercetin interacts with TTA loop. C) Zoomed view of the quercetin intercalation in G-quadruplex DNA groove. D), E), F) RMSD, Electrostatic and VdW energies (200 ns) respectively.

In this study, we conducted MD simulations with a free ligand to analyze the binding modes and interaction features of quercetin and taxifolin to intramolecular human telomeric G-quadruplex structure. Major binding poses were identified and detailed binding pathways were characterized. As a result of comparison analysis between quercetin and taxifolin following conclusions could be stated. One of the most urgent problems in G-quadruplex binding ligands is selectivity. G-quadruplexes binding ligands also bind to B-DNA through intercalation. That explains the lack of selectivity (< 10.000 fold difference required for cancer therapy) of this compounds to two DNA forms. Specificity of G-quadruplex structure topologies allows ligands to have several different binding modes, such as bottom, top, side stacking, interaction with the loops and others. Based on the results of this study, we suggest that designing small molecules or modifying biological ones the way that prevents their intercalation to B-DNA would improve ligands selectivity to a degree. Further investigations of taxifolin and quercetin interaction with other human telomeric G-quadruplex structural forms are required for better understanding of binding patterns of this molecules.

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