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MOLECULAR IDENTIFICATION AND STUDY OF THE ECHOVIRUS EVOLUTION

ABSTRACT

Picornavirus family consists of 6 genera with many pathogenic strains that are for humans. Echoviruses belong to the genus of enteroviruses which comprises of 64 serotypes and is implicated in a wide range of human diseases. Enterovirus serotypes are today classified based on biological and molecular data in 5 species: (i) PV (polioviruses 1-3), (ii) HEV-A (CAV2-8, CAV10, CAV12, CAV14, CAV16, and EV71), iii) HEV-B (CAV9, CBV1-6, all Echoviruses and EV69, iv) HEV-C (CAV1, CAV11, CAV13, CAV15, CAV17-22 and CAV24) and (v) HEV-D (EV68 and EV70). The enteroviral RNA is a single-stranded, positive-sense genome of 7, 4-7, 5 Kb. A small virus encoded protein (VPg) is covalently linked to the 5' terminal nucleotide. Between the 5' and 3' untraslated regions (UTRs) there is a single open reading frame (ORF) of approximately 2100 codons which is translated by a cap dependent mechanism to a long polyprotein. Viral proteases cleave the polyprotein to give the structural and non-structural proteins. The capsid proteins designated VP4, VP3, VP2, VP1 respectively, are encoded by the P1 region. The protease $2A^{pro}$ and proteins 2B, 2C (involved in RNA replication) are encoded by the P2 region. Finally P3 region encodes VPg and precursor 3AB, the major viral protease $3C^{pro}$ and the RNA dependent RNA polymerase $3D^{pol}$.

The classical methods for the detection and characterization of enteroviruses are based on the virus isolation through cell cultures and seroneutralization. Serological diagnosis of enteroviral infections is rather complicated due to the existence of antibody heterotypic reactions, to the lack of a uniformly reacting antigen and to the high serotype number. The development of Reverse Transcription Polymerase Chain Reaction provides with a direct and sensitive tool for the detection of enterovirus genetic material in clinical samples and is used successfully in order to detect those serotypes that are not

propagated in cell culture systems, as well as the non-typable with the classical methods strains.

The aim of the present doctoral thesis was firstly the analysis of different genomic regions of prototype and clinical Echovirus strains as far as it concerns the suitability of them for diagnosis. Then, some of the genome parts were sequenced and noucleotide and phylogenetic relationships were analyzed with the aid of bioinformatics software. The phylogenetic grouping pattern of the clinical isolates revealed a correlation of serotype and genotype either in 5' or in 3' end of VP1 gene but absence of this relationship in the adjacent non-structural genes. The sequences obtained from the 3' part of the genome, enforced the observation that recent echovirus isolates differ significantly from prototype strains in the downstream regions of the genome and provided further evidence that non-structural enterovirus genes are ubiquitous and may combine freely adapting genomic sequences that are not restricted from the place of isolates' origin.