STUDY OF THE IN VITRO PRODUCTION MECHANISM OF POLIOVIRUSES AND EV-C ENTEROVIRUSES RECOMBINANT STRAINS

ABSTRACT

Enteroviruses belong to Picornaviridae family. Their genome is composed of a positive sense single-strand RNA of 7.500bp in length and is surrounded by an icosahedral capsid. Enteroviruses that infect humans are classified into four species: EV-A, EV-B, EV-C and EV-D. Polioviruses, the most significant member of EV-C species, are the causal agents of paralytic poliomyelitis and exist as three distinct serotypes (PV1, PV2 and PV3). Since the 1960s, poliomyelitis has been effectively controlled by the use of two vaccines containing all three serotypes of PV, the inactivated poliovirus vaccine (IPV) and the live attenuated oral poliovirus vaccine (OPV). Despite the success of OPV in polio eradication program, it has shown a significant disadvantage: the emergence of vaccine associated paralytic poliomyelitis (VAPP) and the circulation of vaccine derived polioviruses (VDPVs). VAPP is a result of accumulated mutations and/or recombination events placed at the genome of attenuated vaccine Sabin strains.

The aim of the present thesis was to design and develop different methods in order to study the in vitro production mechanism of recombinant strains of polioviruses and EV-Cs.

During the first part of the thesis a Multiplex-PCR method was designed and developed in order to detect and characterize enteroviruses. The results of this method on prototype and clinical strains proved the ability of the method to stand as a useful tool for fast and accurate detection and characterization of enteroviruses.

At the second part of the thesis, a whole genome sequencing technique, using only four PCR reactions, was designed and developed, using a special primer (DOP), through which a preamplification of entire genome is performed.

During the third part of the thesis, a Multiplex-PCR reaction was designed in order to detect recombination events located from VP1 to 3D genomic region of vaccine derived polioviruses. The results of the Multiplex-PCR proved the ability of the method to detect and identify rare and common recombination types by using only four Multiplex-PCR reactions.

At the fourth part of the thesis a specific Stem-Loop RT-PCR method was designed to detect the replicative activity of enteroviruses through detection of the replicative negative

strand RNA strand. This method was originally applied on Sabin 1 prototype strain, where the replicative activity was correctly detected at high and low virus titres, quite early before the appearance of CPE. Moreover, this method was also used to distinguish between replicative active and inactive CAV prototype strains, that didn't yield CPE in cell cultures.

At the final part of the thesis, the study of recombination events derived from simultaneous Rd cell culture infection using Sabin 1 and CAV13 prototype strains was conducted. Probable secondary RNA structure models were also designed using specific bioinformatics software. The results of this study proved the increased incidence of heterotypic recombination events in 2A or 2B genomic regions and showed that a correlation between recombination sites and secondary RNA structure may be established.