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Targeting Norovirus: Strategies for the Discovery of New Antiviral Drugs

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1. Introduction

Gastroenteritis is globally responsible for great morbidity and mortality among all ages. In the developing countries, it still represents one of the top causes of death for children <5 years of age, resulting in 1,8 million fatalities every year (Boschi-Pinto et al., 2008; Bryce et al., 2005). Viruses are responsible for the majority of cases of gastroenteritis with rotaviruses and noroviruses being the major pathogens.

Noroviruses are today recognized as the leading cause of foodborne outbreaks and sporadic cases of gastroenteritis worldwide (Glass et al., 2009; Patel et al., 2009). Nowadays, they are even considered the second most important agent of severe childhood diarrhea after rotavirus (Koopmans, 2008; Patel et al., 2008; Ramani & Kang, 2009) but the importance of norovirus in this age group is expected to increase in the upcoming years, as a consequence of the implementation of routine rotavirus vaccination (Koo et al., 2010).

The recognition of the clinical importance of norovirus only began in the late 1990s when sensitive routine diagnostic methods became available. In fact, the first norovirus (the *Norwalk* virus) was discovered in 1972 (Kapikian et al., 1972) but more than twenty years were necessary to disclose the important role of these viruses as human pathogens. Noroviruses are now on the upswing and a fundamental question is being raised: are norovirus really emerging? (Widdowson et al., 2005)

Despite the increasing attention given to norovirus today and the significant morbidity and mortality associated with norovirus gastroenteritis, no specific antiviral drugs or vaccines are yet available for treatment or prevention of norovirus illness. Only recently, a recombinant intranasal vaccine has entered a phase I clinical trial (El-Kamary et al., 2010; Vinje, 2010)

This chapter will highlight the importance of finding specific antiviral therapy for norovirus infection and explore biological features of norovirus, pointing out directions to stop or control this important human pathogen.

1.1 Clinical disease, transmission and epidemiology

Norovirus gastroenteritis is generally acute and self-limited, but in infants, elderly, and immunocompromised individuals it may be more severe and prolonged since they are more

susceptible to complications due to dehydration (Green, 2007; Patel et al., 2008). After an incubation period of 24–48 h, there is an acute onset of symptoms of nausea, vomiting, abdominal cramps, myalgias, and intense non-bloody diarrhea which usually resolves in 2–3 days (Green, 2007). The median duration of illness can be longer, lasting up to six weeks in infants and young children (Kirkwood & Streitberg, 2008; Murata et al., 2007; Patel et al., 2009). Prolonged shedding is also documented in transplant patients and other immunosuppressed individuals, with symptoms lasting over two years (Hutson et al., 2004; Widdowson et al., 2005). Deaths have been reported in elderly during outbreaks in nursing homes, hospitals and cruise ships (Gotz et al., 2002; Lopman et al., 2004; Patel et al., 2008). Repeated associations of norovirus infections with clinical outcomes other than gastroenteritis have been reported (CDC, 2002; Chen et al., 2009; Kawano et al., 2007; Marshall et al., 2007; Turcios-Ruiz et al., 2008). Moreover, norovirus RNA has been detected in the blood of children with norovirus gastroenteritis and in the cerebrospinal fluid of a child with encephalopathy (Ito et al., 2006; Takanashi et al., 2009). This data suggests that norovirus infection is probably not limited to the intestine and could disseminate to systemic sites.

Noroviruses are transmitted by the fecal-oral route either directly from person-to-person or indirectly through consumption of contaminated food (fresh fruit, vegetables, shellfish and bakery products), water (drinking, ice or swimming) or following exposure to contaminated environmental surfaces and to airborne vomitus droplets (Green, 2007). Concerns about a potential zoonotic transmission have been raised given the close genetic relatedness between norovirus found in humans and animals and the presence of antibodies to animal strains in humans (Bank-Wolf et al., 2010).

Norovirus outbreaks are notably extensive and often occur in semi-closed environments (nursing homes, hospitals, day-care centers, schools, cruise ships and restaurants) that favor person-to-person transmission (Glass et al., 2009; Patel et al., 2009). Moreover, modern lifestyles make people more vulnerable to norovirus. More elderly people and infants live in communal settings, people eat more food outside the household (handled by potentially infected workers), consume more imported fresh fruit and vegetables from countries where crops are still irrigated with sewage-contaminated water and also more people are travelling and being exposed to norovirus in hotels, airplanes and cruise ships (Widdowson et al., 2005).

The very low infectious dose of norovirus (≈ 17 virus particles), their long persistence in the environment, withstanding sanitary measures effective against other microorganisms (freezing, heating and chlorination) combined with prolonged asymptomatic viral shedding make norovirus extremely infectious and explain the extensiveness of outbreaks (Duizer et al., 2004a; Siebenga et al., 2008; Teunis et al., 2008; Widdowson et al., 2005). Additionally, repeated infections can occur throughout life with re-exposure, likely due to the lack of lasting immunity and the lack of complete cross-protection against the diverse norovirus strains. (Donaldson et al., 2010; Patel et al., 2009).

Over the past years, a global increase in the number of norovirus outbreaks was noticed, with the GII.4 variants being accountable for the vast majority of cases. These GII.4 variants have become globally predominant and were responsible for four pandemics in the last two decades (Lindesmith et al., 2008; Siebenga et al., 2008). This emergence of dominants strains

of norovirus causing worldwide epidemics suggests a pattern of epochal evolution resembling that of influenza (Glass et al., 2009).

1.2 Host susceptibility and virus-host interaction

Susceptibility to norovirus infection involves both acquired immunity and genetic resistance (Parrino et al., 1977). Volunteer studies found that some individuals were repeatedly susceptible to norovirus infection whereas others were repeatedly resistant (Parrino et al., 1977). Although it was initially unclear why some subjects did not develop illness, current research suggests that host genotype is a prominent factor in the development of norovirus infection since it depends on the presence of specific human histo-blood group antigen (HBGA) receptors in the gut of susceptible hosts (Lindesmith et al., 2003). Infection by norovirus relies on the recognition of HBGAs in the initial viral attachment and this key event most likely controls host susceptibility and resistance to norovirus (Hutson et al., 2004; Marionneau et al., 2002; Tan & Jiang, 2005, 2011).

HBGAs are complex carbohydrates linked to proteins or lipids on the surface of red blood cells and mucosal epithelia of the respiratory, genitourinary and digestive tracts, or present as free oligosaccharide in biological fluids such as milk and saliva. These antigens provide diversity within the human population and their biosynthesis is controlled by the enzyme products of alleles at the ABH, fucosyltransferase (FUT) 2, and FUT 3 loci (Hutson et al., 2004).

A number of distinct binding patterns of noroviruses to HBGAs have been described according to the ABO, Lewis and secretor types of the human HBGAs (Huang et al., 2003; Huang et al., 2005). This explains the correlation between secretor status and susceptibility to Norwalk virus infection, where secretor individuals with a wild-type *FUT2* gene (~80% of the population), who express HBGAs on gut epithelial cells and in body fluids, are susceptible to Norwalk virus infection, while nonsecretors, with a null *FUT2* allele, are completely resistant (Lindesmith et al., 2003).

The binding patterns of noroviruses to HBGAs are currently sorted into three major groups, the H, the A/B, and the Lewis binding groups (Tan & Jiang, 2011). While norovirus strains display distinct HBGA binding properties, collectively they can infect nearly all individuals due to their high genetic variability (Le Pendu et al., 2006). This highlights the highly adaptive nature of noroviruses and the likelihood of a long co-evolution of human noroviruses with their human host.

1.3 Classification and genome organization

Noroviruses are a genetically diverse group of viruses belonging to the genus *Norovirus* of the family *Caliciviridae* (Green, 2007). The Norwalk virus was the first norovirus to be discovered and associated to a gastroenteritis outbreak in an elementary school in Norwalk, Ohio, USA in 1968 and is today considered the prototype of the genus *Norovirus* (Kapikian et al., 1972).

The family *Caliciviridae* comprises, besides *Norovirus*, four accepted (*Sapovirus, Lagovirus*, *Vesivirus, Nebovirus)* and two tentative genera (*Recovirus, Valovirus*), that include human and non-human pathogenic viruses (Farkas et al., 2008; Green, 2007; Green et al., 2000; L'Homme

et al., 2009). The two genera *Norovirus* (NoV) and *Sapovirus* (SaV) are the only that comprise human pathogenic agents, both causing acute gastroenteritis. The other genera include important veterinary pathogens such as the rabbit hemorrhagic disease virus (RHDV) which causes an often fatal hemorrhagic disease in rabbits in the genera *Lagovirus*, the feline calicivirus (FCV) which causes a respiratory disease in domestic and wild cat species in the genera *Vesivirus*, and the Newbury-1 virus which infect bovines in the genera *Nebovirus.* The tentative genera *Recovirus,* comprises the Tulane virus (TV) isolated from stool samples of rhesus macaques whose pathogenicity remains to be elucidated (Farkas et al., 2008). The other tentative genera *Valovirus* comprises the St-Valérien-like viruses isolated from pig feces (L'Homme et al., 2009).

Noroviruses are today classified into five genogroups (GI-V) divided into at least 31 genetic clusters or genotypes based on sequence diversity in the complete capsid protein VP 1 (Zheng et al., 2006). Genogroups share > 60% amino acid identity in the VP 1 and each genetic cluster or genotype shares > 80% identity in amino acid sequence of VP 1 (Green et al., 2000; Zheng et al., 2006). Human noroviruses have been associated with GI, GII and GIV and bovine and murine noroviruses belong to GIII and GV, respectively (Wobus et al., 2004; Zheng et al., 2006). GII also contains swine strains and GIV comprises the feline (lion) and canine strains (Martella et al., 2007; Martella et al., 2008; Mesquita et al., 2010).

Noroviruses are small icosahedric non-enveloped viruses of 27-32 nm with a positive-sense single stranded (ss) RNA genome of 7.4–7.7 kb, organized into three open reading frames (ORF1-3) (Fig 1). The 5′ proximal region of the norovirus genome encodes a polyprotein of six/seven nonstructural protein products in a single ORF (ORF1). ORF2 encodes the major structural capsid protein VP1 and ORF3 the minor structural protein VP2. Norovirus genome is covalently linked, at the 5′ end, to a viral protein called VPg (virion protein, genome-linked) and is polyadenlyated at the 3′ end (Green, 2007).

The ORF1 of norovirus encodes the six/seven nonstructural proteins in the following order: the p48/ N-terminal protein (or NS1-2), the NTPase (NS3), the p22 (NS4), the VPg (NS5), the viral protease (Pro, NS6), and the viral RNA-dependent RNA polymerase (RdRp, NS7). Some of these proteins have defined activities such as NS3, an NTPase (nucleoside triphosphatase) (Pfister & Wimmer, 2001), NS6, a protease (Liu et al., 1996), and NS7, an RNA-dependent RNA polymerase (RdRp) (Fukushi et al., 2004; Rohayem et al., 2006a). Also, NS5 known as VPg, is a protein that is covalently attached to the 5′ ends of viral genomes in place of a typical 5′ cap and that can function as a primer in viral RNA replication (Burroughs & Brown, 1978; Rohayem et al., 2006b). The role of the remaining nonstructural proteins (NS1-2 and NS4) in norovirus replication is not yet well defined (Green, 2007). Available data suggests that NS1-2 and NS4, also known as N-term/p48 and p22, respectively, both contribute to norovirus replication complex formation on intracellular membranes, including that of the Golgi apparatus, and disrupt intracellular host protein trafficking (Ettayebi & Hardy, 2003; Fernandez-Vega et al., 2004; Hyde et al., 2009).

The major structural protein of norovirus, the VP1, is encoded by ORF2. Virions contain 180 copies or 90 dimers of VP1 that assemble into icosahedral particles (Prasad et al., 1999; Prasad et al., 1994). The VP1 protein is divided into a conserved internal shell domain (S) and a more variable protruding domain, the P domain, that forms the arch-like protrusions and is further subdivided into P1 and P2 domains (Fig. 1). The P2 subdomain is located at

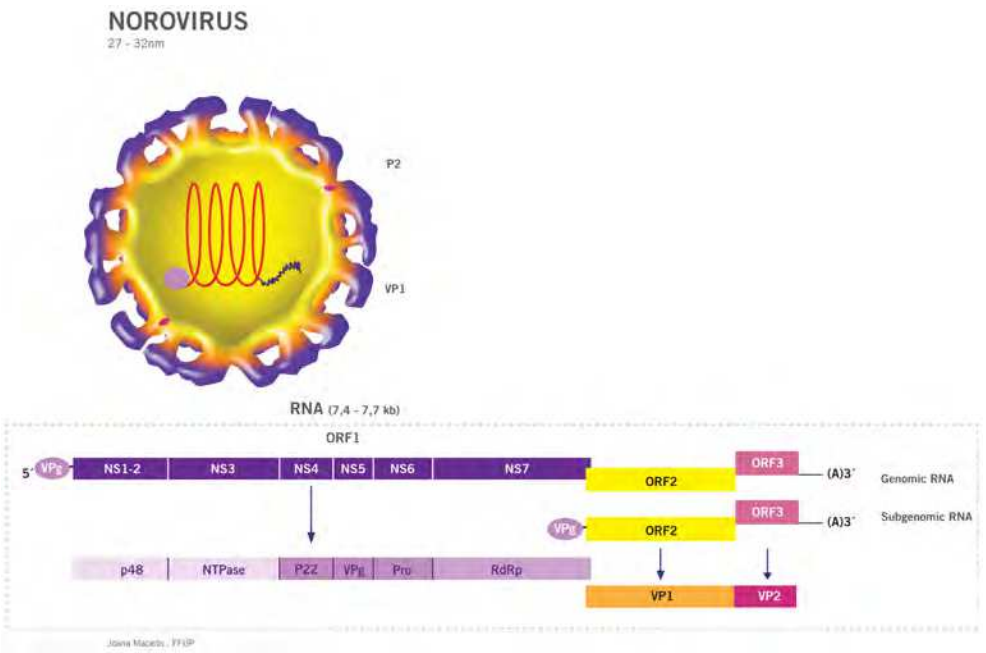

Fig. 1. Schematic representation of a viral particle and genome organization of norovirus. Genomic and subgenomic RNA of norovirus with the genome linked protein VPg at the 5′ end and the poly(A) tail at 3′ end is shown along with the nonstructural proteins (NS1-7) encoded by ORF1 as well as the structural proteins (VP1 and VP2), encoded by ORF2 and 3. See text for further details

the outmost surface of the viral capsid and comprises a hypervariable region, where resides the binding interface for HBGA association with norovirus (Bu et al., 2008; Cao et al., 2007; Choi et al., 2008; Tan et al., 2003). The VP2 is a small, basic structural protein encoded by the ORF3 which is present in one or two copies per virion (Glass et al., 2000; Hardy, 2005). Its function in viral replication is currently undefined but there is evidence that it increases the level of expression of VP1 and stabilizes virus-like particles (VLPs, generated through expression of the VP1) (Bertolotti-Ciarlet et al., 2003). The basic charge of VP2 suggests that it may function in encapsidation of the viral genome (Karst, 2010).

1.4 Replication of norovirus

The replication of norovirus has not been yet fully elucidated and most of the current knowledge is drawn by analogy with other (+)ssRNA viruses and studies with related animal caliciviruses. Norovirus is one of the positive-sense ssRNA viruses whose genome functions directly as the mRNA, beginning the infectious cycle with the synthesis of a precursor that only gives rise to the nonstructural proteins, including the RdRp enzyme that transcribes then one subgenomic mRNA encoding the structural proteins (VP1 and VP2) (Green, 2007). Like the other (+)ssRNA viruses , the replication of norovirus occurs in the cytoplasm. In Fig 2 is represented a scheme of the replication of norovirus.

In the first step, the viral attachment of the virion to the cell receptor, the P2 subdomain of the VP1 binds to a sugar residue, mostly to the HBGA carbohydrates in the case of human noroviruses but also to sialic acid or heparan sulfate (Hutson et al., 2002; Marionneau et al., 2002; Rydell et al., 2009; Stuart & Brown, 2007; Tamura et al., 2004; Taube et al., 2009). This interaction between VP1 and HBGA seems not to be enough for the entry of norovirus in host cells and the involvement of a membrane protein as a receptor or co-receptor for subsequent penetration/entry is suspected (Tan & Jiang, 2010).

Norovirus enters the cell using a non-clathrin-, non-caveolin-mediated endocytic pathway but dependent on dynamin II and cholesterol (Gerondopoulos et al., 2010; Perry & Wobus, 2010). Moreover, this entry step is pH-independent and no conformational changes in the capsid required for viral uncoating are observed with acidic intracellular pH (Perry et al., 2009).

After the internalization in the cell and uncoating of viral genome, the translation of ORF1 of the viral genomic RNA produces a large protein, the so called nonstructural polyprotein. The initiation of translation is dependent on the interaction of the VPg with the cellular translation initiation machinery (Daughenbaugh et al., 2003; Daughenbaugh et al., 2006). A co-translational processing releases the nonstructural proteins and their precursors (Sosnovtsev, 2010). The proteolytic processing is mediated by the viral protease (NS6) which is autocatalytically released from the polyprotein precursor (Putics et al., 2010).

The replication of norovirus is believed to occur in a replication complex (RC) formed by intracellular membranous structures which contain all the viral nonstructural proteins along with host proteins that will help viral replication as well as the viral RNA intermediate, ssRNA and double stranded RNA (dsRNA) (Hyde & Mackenzie, 2010; Hyde et al., 2009). The recruitment of host membranes (RE, Golgi, endossomes) necessary for the formation of the RC, is induced by the viral non structural proteins p48 (NS1-2) and p22 (NS4), through a modulation of the host cell secretory pathway (Denison, 2008; Hyde & Mackenzie, 2010; Sharp et al., 2010).

Once the RC is assembled, the RdRp (NS7) starts the synthesis of the antigenomic RNA (negative sense) from the genomic (positive sense) RNA template. The initiation of antigenomic RNA synthesis by the RdRp is dependent upon uridylylation of VPg that serves as a primer in the presence of the polyadenylated genomic RNA (Rohayem et al., 2006a; Rohayem et al., 2006b). This antigenomic RNA is then used as a template for synthesis of the new genomic RNA and of the subgenomic RNA.

The newly synthesized genomic RNA is either translated as a polyprotein precursor or used for packaging in the assembled viral protein core. The subgenomic RNA (positive sense) is translated as structural proteins, VP1 and VP2. Finally, the structural proteins are assembled and the genomic RNA packaged, followed by release of the mature virion from the cell. This late stages of replication are, however, poorly understood.

1.5 Surrogate models for the study of human norovirus

Human noroviruses are not cultivable in routine laboratory cell culture or primary tissue cultures (Duizer et al., 2004b). There has been a single report using a 3-D cell culture system demonstrated for the first time successful passage of both GI and GII norovirus *in vitro*

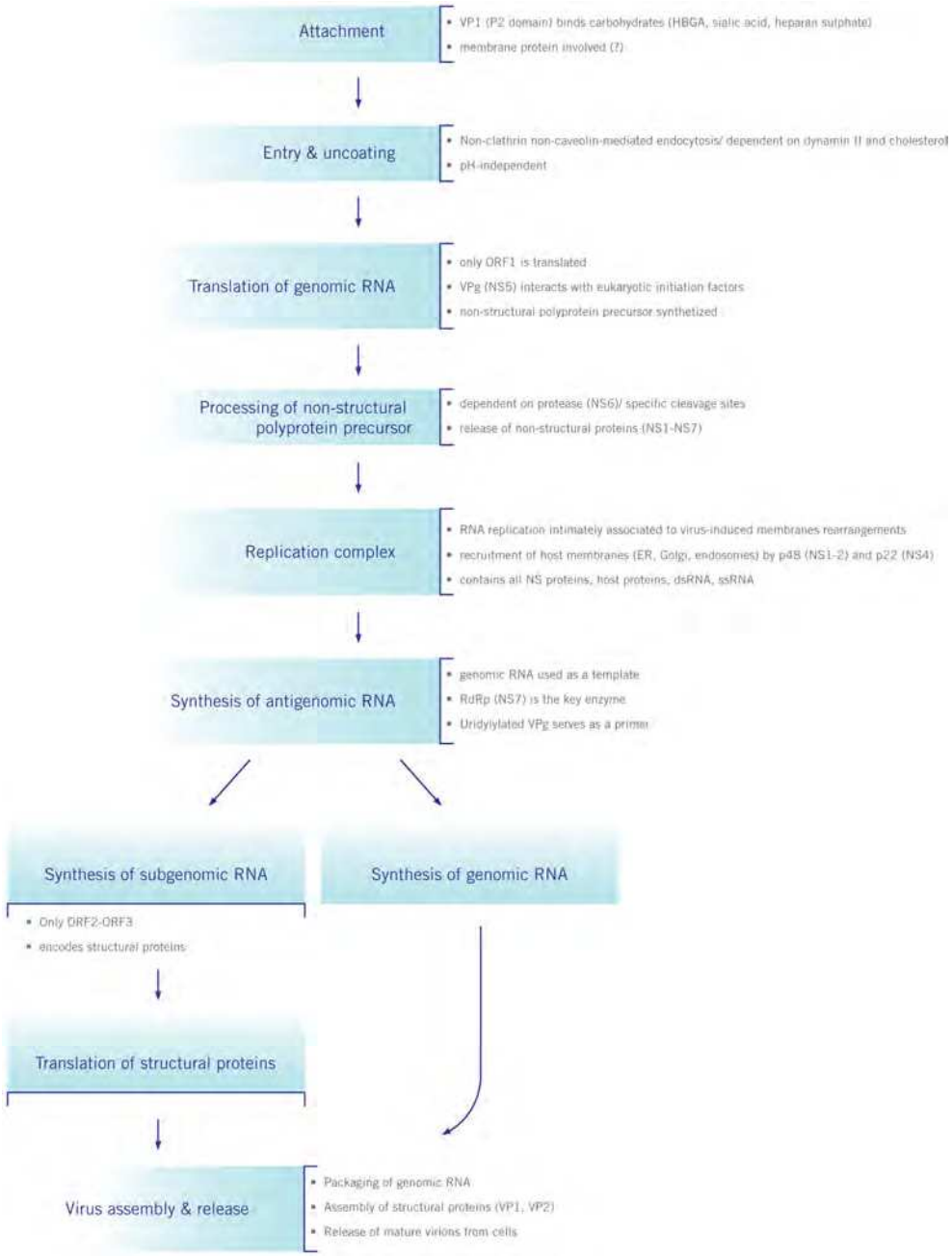

Fig. 2. Replication scheme of norovirus and key events.

(Straub et al., 2007) but several independent laboratories failed to reproduce such results (Papafragkou et al., 2009). For this reason, the study of human norovirus has been made using surrogate viruses.

Initially, surrogate models for norovirus infectivity included viruses from other genera within the family *Caliciviridae,* namely porcine enteric calicivirus, a *Sapovirus*, and the feline calicivirus (FCV), a *Vesivirus* (Doultree et al., 1999; Duizer et al., 2004a) (Fig.3).

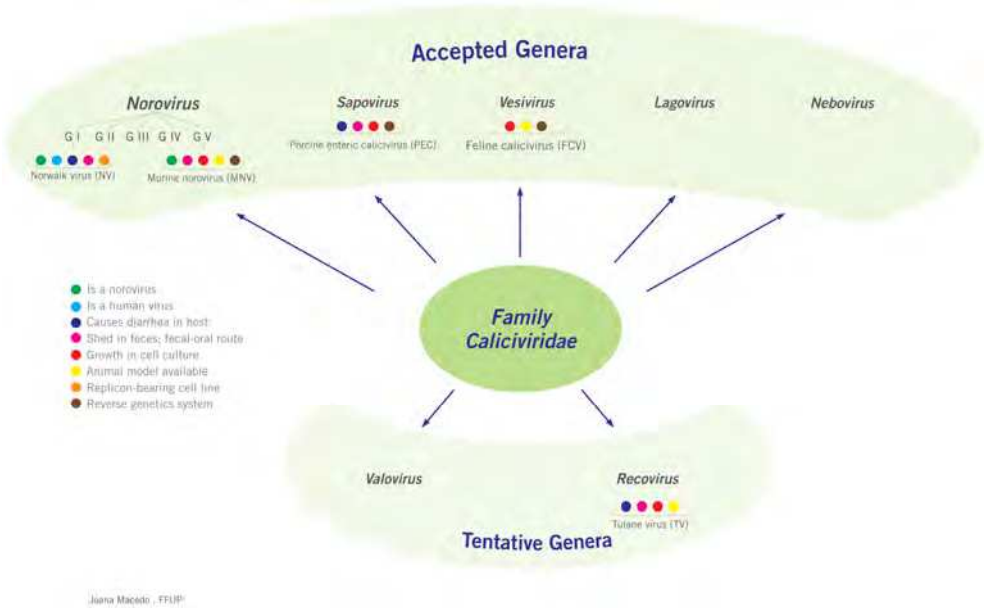

Fig. 3. Characteristics of the different surrogates models for human norovirus within the genera of the family C*aliciviridae*

FCV was then frequently used as a surrogate model for survival, persistence and inactivation studies of human noroviruses until the more recent discovery of murine norovirus (MNV) (Bae & Schwab, 2008; Cannon et al., 2006; Wobus et al., 2004; Wobus et al., 2006). MNV is a genogroup V norovirus and is to date the only *Norovirus* able to replicate both in cell culture and in a small animal model. Moreover, like human norovirus MNV is an enteric pathogen that spreads through the fecal-oral route and is shed at high levels in the feces (Karst et al., 2003; Wobus et al., 2004). On the other hand, the use of FCV was criticized because: (i) it does not belong to the genus *Norovirus*; (ii) it is a respiratory virus; (iii) it is not shed in feces and (iv) it cannot survive at low pH, a necessary characteristic of enteric viruses that must survive passage through the stomach (Bae & Schwab, 2008; Cannon et al., 2006; Duizer et al., 2004b; Wobus et al., 2006). Therefore, MNV is considered today the best surrogate model for human norovirus (Wobus et al., 2004; Wobus et al., 2006).

MNV has the size, shape, and buoyant density characteristic of human norovirus (Green, 2007; Karst et al., 2003). MNV genome has also the three ORFs characteristic of noroviruses (Wobus et al., 2006), but it has an additional ORF4 that overlaps ORF2 in a different reading

frame (Thackray et al., 2007). ORF1 of MNV also encodes the nonstructural proteins and ORFs 2 and 3 encode the two proteins of the the viral capsid (Sosnovtsev et al., 2006). The conserved molecular features of MNV and human norovirus genomes suggest that many fundamental mechanisms of replication are conserved between murine and human noroviruses (Wobus et al., 2006). Although MNV infection seems to be asymptomatic in immunocompetent mice, it causes diarrhea and lethality in mice deficient in components of innate immunity (Karst et al., 2003; Mumphrey et al., 2007). Hence, the clinical presentation of MNV is different from the one of human norovirus, and for this reason MNV does not constitute yet the ideal model for studying human norovirus.

The recent discovery of the Tulane virus (TV), a novel calicivirus isolated from stool samples of rhesus macaques, together with the likelihood of this virus causing intestinal infection and the availability of a tissue culture system could make TV a valuable surrogate for human norovirus (Farkas et al., 2008).

An alternative approach to the study of norovirus RNA replication was established when a human norovirus replicon-bearing cell line was created by transfecting a plasmid containing most of the Norwalk virus genome into mammalian cell lines (Chang et al., 2006). These cell lines are capable of constitutively expressing the replicative enzymes and other nonstructural proteins, allowing the study of RNA replication and providing a platform for screening antiviral compounds.

Reverse genetics systems have been developed for some caliciviruses, namely for PEC, FCV, MNV and TV (Chang et al., 2005; Chaudhry et al., 2007; Sosnovtsev & Green, 1995; Ward et al., 2007; Wei et al., 2008; Wobus et al., 2004). These systems have helped to elucidate fundamental aspects of caliciviruses replication through the introduction of deliberate changes in certain genes and the analysis of the resultant effect in the virus phenotype. Reverse genetics systems represent also an important tool for the development of antivirals against norovirus (Putics et al., 2010).

Virus-like particles (VLPs) which result of the independent expression and self-assembly of the VP1 are also valuable tools that have been used in many research areas of norovirus (El-Kamary et al., 2010; Jiang et al., 1992). These VLPs are morphologically and antigenically indistinguishable from the native forms of viruses found in human stools, and retain the binding properties of native norovirus virions at least in terms of carbohydrate association (Jiang et al., 1992). VLPs have been used for the development of immunological assays, the study of virus-host interaction, structural studies of the norovirus capsid, investigation of antigenic relationships and as potential vaccine candidates (Green, 2007; Tan & Jiang, 2005).

2. Strategies to inhibit the replication of norovirus

The antiviral research of norovirus is still in its infancy and there are only few reports of antivirals for norovirus. Recently, a new chemical scaffold, the 2-styrylchromones, has shown anti-norovirus activity in the MNV surrogate model, opening the door to the search of anti-norovirus drugs among a wider range of novel compounds from different chemical families (Rocha-Pereira et al., 2010). Additionally, nitoxanide, a prodrug used to treat protozoal gastroenteritis has been reported to reduce the duration of norovirus gastroenteritis in a clinical trial although the mechanism of action remains unclear (Rossignol & El-Gohary, 2006).

In the following section, potential targets and strategies to inhibit norovirus life cycle are presented, based on the information available today on norovirus genome organization, functions of structural and nonstructural proteins, replication and virus-host interaction (Fig. 4). The predictable advantages and/or disadvantages of these strategies are discussed and some antiviral drugs previously identified as active against similar molecular targets in other plus stranded RNA viruses are suggested as a starting point.

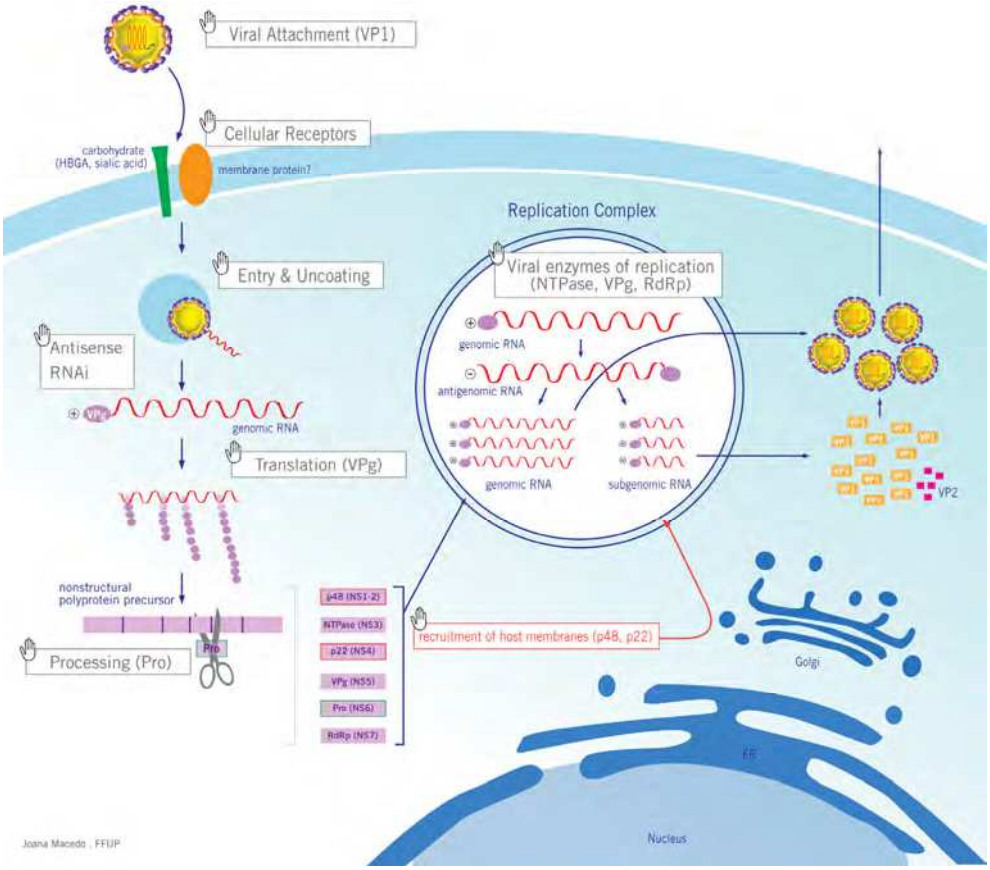

Fig. 4. Targeting the various steps of the replication of norovirus

2.1 Targeting cellular receptors of norovirus

Human noroviruses recognize HBGA carbohydrates present on cell surface, which are key players in the initial viral attachment, acting most likely as cellular receptors or co-receptors of norovirus (Hutson et al., 2002; Marionneau et al., 2002; Tan & Jiang, 2005, 2011)

Noroviruses present diverse binding patterns to HBGAs, being currently sorted into three major binding groups, the H, the A/B, and the Lewis binding group (Tan & Jiang, 2011). The interaction between noroviruses and HBGA is highly strain-specific rather than genogroup- or genotype-specific (Tan & Jiang, 2010). Hence, strains of the H, A/B and

Lewis binding groups can be found in both two major genogroups of human noroviruses (GI and GII).

The existence of only three HBGA-binding interfaces makes possible the design of antivirals against these targets in noroviruses. Any compound that targets a given HBGA binding interface may be capable of blocking infection of all strains that bind that same type of HBGA. Thus, only three different HBGA-binding interfaces would need to be targeted by compounds to block nearly all noroviruses in the GI and GII genogroups (Tan et al., 2009).

The strategy of targeting this first step of virus-receptor interaction could be of great interest to use as prophylactic therapy since it would be effective in preventing infections of individuals in high risk settings or that were in contact with an index case during an outbreak (Tan & Jiang, 2008). Furthermore, it would be expected that such compounds would be able to reduce the severity of symptoms in already infected individuals and likely reduce virus excretion, limiting its propagation to higher numbers of individuals (Tan & Jiang, 2008). Citrate, and other glycomimetics, showed to have the potential to block human noroviruses from binding to HBGAs (Hansman et al., 2011) providing a starting point for norovirus inhibitors.

However, there are predictable difficulties to this strategy, one of which being the fact that the early steps of norovirus life cycle are not yet fully disclosed. Recent findings of FCV and MNV binding to sialic acid (Rydell et al., 2009; Stuart & Brown, 2007; Taube et al., 2009) or heparan sulfate (Tamura et al., 2004) broadens the spectrum of sugar residues that interact with these viruses. Moreover, the identification of a membrane protein, the junctional adhesion molecule A (JAM-A), as a receptor for FCV (Makino et al., 2006) raises the hypothesis of this protein or other members of the Ig superfamily being also cellular receptors for caliciviruses, like they are for reoviruses and picornaviruses (Tan & Jiang, 2010). According to the proposed model for reoviruses, the virus interact firstly with a sugar residue like sialic acid, as a determinant of tropism that is responsible for initial virus attachment, and later use a membrane protein (JAM-1) to enter the host cell (Barton et al., 2001). The role of these two molecules in viral attachment and entry is likely to occur also in FCV, and one could even speculate that it could be extended to noroviruses since it is usual that viruses within the same family use similar cellular receptors (Tan & Jiang, 2010).

Much remains to be unraveled in this field, therefore there are still doubts about the selection of the best target for chemical compounds to block or interfere with virus attachment.

2.2 Targeting entry and uncoating of norovirus

In order to enter host cells, viruses take advantage of cellular processes entering by an endocytic pathway, most commonly a clathrin-mediated endocytosis. Viral entry can also occur via caveolin-mediated endocytosis, clathrin/caveolin-independent endocytosis, macropinocytosis, or phagocytosis (Marsh & Helenius, 2006). After entrance in the host cell, the uncoating of virus must occur in order to deliver the viral genome into the host cytoplasm. This event is often triggered by the acidic environment of endosomes but it can also occur after the binding of virus to cellular receptors (Tsai, 2007).

Studies with FCV showed this virus enters cells by clathrin-mediated endocytosis in a pH-dependent manner (Stuart & Brown, 2007). However, it was demonstrated that the entry of MNV is clathrin/caveolin-independent but mediated by dynamin II and cholesterol (Gerondopoulos et al., 2010; Perry & Wobus, 2010). In addition, studies with MNV show this

virus is pH-independent and that a low intracellular pH does not trigger conformational changes in the capsid required for MNV uncoating (Perry et al., 2009). This difference in sensitivity to low pH between FCV and MNV was suggested to be related with the different routes of infection of these viruses (Perry et al., 2009). While MNV is an enteric virus that infects its host by the small intestine and retains infectivity for hours at a pH of 2 (similarly to the human Norwalk virus), FCV is a respiratory virus that significantly decreases infectivity at low pH (Cannon et al., 2006; Dolin et al., 1972).

Further understanding of the cellular mechanisms of norovirus entry and uncoating would bring out new antiviral targets.

2.3 Targeting structural proteins of norovirus

The VP1 is the major structural protein of norovirus and its P2 subdomain, which is located at the outmost surface of the viral capsid, comprises the binding surface for HBGA (Bu et al., 2008; Cao et al., 2007; Choi et al., 2008; Tan et al., 2003). The function of VP2 is currently undefined and there is not sufficient information to address this minor protein as an antiviral target.

The search of compounds targeting VP1 of norovirus was explored through a saliva-based enzyme immunoassay (EIA) that measures their capacity to block the binding of norovirus VLPs to HBGAs present in such biological fluid (Feng & Jiang, 2007). Different chemical compounds were found to be strong inhibitors of this binding being potential candidates for further development as antivirals for norovirus (Feng & Jiang, 2007), but one should be aware that a saliva-based EIA was used instead of a cellular system or animal model and this could be regarded as an handicap.

Antiviral drugs that target viral surface proteins of other RNA virus have been described, such as pleconaril, a picornavirus capsid-binding compound, but resulted in a not entirely successfully strategy (Field & Vere Hodge, 2008). The problem with this kind of drugs relies on their low genetic barrier since the viral surface proteins can undergo variations without compromising viral fitness, easily allowing resistant strains to emerge (Field & Vere Hodge, 2008). The same problem would most likely take place with norovirus given its well known antigenic variation. A strategy could be the use of combination therapy of this class of compounds together with drugs against well conserved nonstructural proteins with critical functions. This would avoid or at least delay the development of resistance.

2.4 Targeting nonstructural proteins

The six/seven nonstructural proteins of norovirus are the p48/ N-terminal protein (NS1-2), the NTPase (NS3), the p22 (NS4), the VPg (NS5), the viral protease (Pro, NS6), and the viral RNA-dependent RNA polymerase (RdRp, NS7). Since noroviruses share some similarities with picornaviruses, the nomenclature and functions of these ORF1-encoded proteins of norovirus was initially predicted through comparative sequence analysis of their picornavirus counterparts. Hence, the norovirus RdRp was called 3D-like, the protease was called 3C-like, the p22 was called 3A-like, the NTPase was named 2C-like and the p48/N-term was related to the 2B protein (Green, 2007). This resemblance could be important for the search of antivirals against norovirus since the known strategies and targets for inhibiting the replication of picornavirus might be also effective for norovirus.

Although a detailed knowledge of the role of nonstructural proteins is still not available, some important clues have raised from studies with MNV which showed that all these proteins play a role in norovirus replication and are associated with the replication complex (RC) and the viral RNA intermediate dsRNA (Hyde et al., 2009). There is also evidence that the replication complex of MNV is associated with host membranes, namely of the endoplasmic reticulum (ER), the Golgi apparatus and endossomes which suffer virus-induced rearrangements (Hyde et al., 2009; Wobus et al., 2004). These and other features of each nonstructural protein are described below, as well as how these could be used for the discovery of antiviral drugs against norovirus.

2.4.1 p48

The role of p48 or NS1-2 is not yet well defined but it is thought to be comparable to that of the analogous picornavirus 2B protein, which participates in intracellular membrane changes that occur during virus replication (Sosnovtsev, 2010). Studies with Norwalk virus indicated that the p48 may interfere with disassembly of the Golgi complex and cellular protein trafficking (Ettayebi & Hardy, 2003; Fernandez-Vega et al., 2004) while studies with MNV and FCV point out that p48 is associated with the recruitment of ER membranes to the RC (Bailey et al., 2009; Hyde & Mackenzie, 2010). This recruitment of membranes is vital to the synthesis of new viral proteins by the actively replicating noroviruses (Hyde et al., 2009; Wobus et al., 2004). Hence, the impairment of the mechanisms controlled by p48 in the membrane rearrangements could be a good strategy to stop the norovirus life cycle.

2.4.2 NTPase

The NTPase or NS3 shares sequence motifs with other viral NTPases, namely the picornaviruses 2C protein and the flaviviruses NS3 helicase/NTPase. It has also been classified in the superfamily 3 of RNA helicases (Hardy, 2005; Pfister & Wimmer, 2001). The role of these enzymes consists in catalyzing the hydrolysis reaction of nucleoside triphosphates and using the released energy to unwind the viral nucleic acids (Kwong et al., 2005). However, until now there is only experimental data confirming the NTPase but not the helicase activity of this nonstructural protein of norovirus (Hardy, 2005).

In recent years, substantial efforts have been made to identify inhibitors of such proteins, since they are well conserved and essential for viral replication. The thiazolobenzimidazoles were identified as potent inhibitors of picornaviruses, showing one of these compounds, TBZE-029 [1-(2,6-difluorophenyl)-6-trifluoromethyl-1*H*,3*H*-thiazolo[3,4-a]benzimidazole] to target the protein 2C of coxsackievirus B3 (De Palma et al., 2008a; De Palma et al., 2008c). These and other picornavirus 2C-targeting compounds, such as MRL-1237 [1-(4-fluorophenyl)-2-(4-imino-1,4-dihydropyridin-1-yl) methylbenzimidazole hydrochloride] and HBB [2-(α-hydroxybenzyl)-benzimidazole] (De Palma et al., 2008a; De Palma et al., 2008c; Norder et al., 2011) could as well be inhibitors of the NTPase of norovirus. The multiple mechanisms through which compounds could inhibit the helicase/NTPase have been extensively described elsewhere (Rohayem et al., 2010). The search of norovirus NTPase inhibitors could result in the development of an innovative antiviral strategy and at the same time reveal functional details of this enzyme of norovirus.

2.4.3 p22

The function of p22 or NS4 in norovirus life cycle is also not yet fully understood. This protein occupies in norovirus genome a position similar to that of the 3A protein in picornavirus genomes but they share only limited sequence similarity (Green, 2007; Hardy, 2005). The 3A protein is known to inhibit the protein trafficking from the ER to Golgi apparatus, a function central to the cellular homeostasis (Wessels et al., 2006). A similar function has been recently described for Norwalk virus p22 (Sharp et al., 2010). It was reported that this protein contains a well-conserved motif that mimics the normal ER export signal, leading to the inhibition of protein trafficking to Golgi, inducing its disassembly and ultimately inhibiting cellular protein secretion (Sharp et al., 2010). Since this motif is highly conserved in human noroviruses, it may constitute a new target for the design of anti-norovirus drugs (Sharp et al., 2010).

Besides, based on what has been described for picornavirus 3A protein, one of the consequences of the inhibition of cellular protein secretion by p22 could be a more severe infection due to the decrease of surface MHC class I proteins and normal cytokine release that leads to a reduction of clearance in infected cells (Sharp et al., 2010; Wessels et al., 2006).

Additionally, there is evidence suggesting that p22 is part of the norovirus RC participating in the recruitment of membranes from the late secretory pathway (Golgi and endossomes) to the RC, being the main viral protein known to be responsible for the recruitment of endossome membranes (Hyde & Mackenzie, 2010).

Two compounds, enviroxime and TTP-8307, have shown to inhibit the *in vitro* replication of enteroviruses and rhinoviruses by targeting the nonstructural protein 3A (De Palma et al., 2009; Heinz & Vance, 1995). Therefore, these compounds are good candidates to be evaluated for their ability to inhibit p22 and the norovirus replication.

2.4.4 VPg

The VPg or NS5 is covalently linked to the 5'-end of the norovirus RNA genome and plays an important role in the replication of norovirus. Initiation of RNA synthesis by viral RdRp is dependent upon uridylylation of VPg that serves as a primer in the presence of the polyadenylated genomic RNA (Rohayem et al., 2006a; Rohayem et al., 2006b). This "protein-primed" initiation occurs after the annealing of the elongated VPg-poly(U) to the poly(A) tail of the viral genome (Rohayem et al., 2006a; Rohayem et al., 2006b).

The inhibition of this uridylylation step or prevention of annealing to the poly(A) tail through a disruption of interaction between VPg and the viral genome or the RdRp could be an effective manner to stop the norovirus life cycle and therefore a good antiviral strategy (Rohayem et al., 2010).

It is known that the initiation of translation of norovirus proteins is dependent on the interaction of the VPg with eIF4F complex (eukaryotic initiation factor 4F), a key component of the cellular translation initiation machinery (Daughenbaugh et al., 2003; Daughenbaugh et al., 2006). The interaction of the VPg with individual eIF4F components has been described. A direct interaction with eIF4E (cap binding protein) was firstly demonstrated with the VPg of Norwalk virus (Goodfellow et al., 2005). A similar interaction with this component was also seen with FCV and MNV but, in these viruses, the key requirement for

RNA translation was shown to be the interaction with another component of the complex, the eIF4A (a RNA helicase) (Chaudhry et al., 2006). This was demonstrated by the inhibitor of eIF4A hippuristanol which blocked the translation of both FCV and MNV viral proteins *in vitro* (Chaudhry et al., 2006). However, the potential use of hippuristanol in therapy is limited by the fact that this compound is also an inhibitor of cellular protein synthesis, therefore expected to be cytotoxic. Panteamine is another compound that has the potential of interfering with VPg/ eIF4F complex, since it stimulates the helicase/NTPase activity of eIF4A, dysregulating its function within the eIF4F complex (Bordeleau et al., 2006). Therefore, these classes of compounds could potentially be explored as antivirals for norovirus but with less cytotoxic derivatives.

2.4.5 Protease

The norovirus protease or NS6 is a cysteine protease that is responsible for processing the nonstructural polyprotein precursor into the six/seven nonstructural proteins (Green, 2007). The atomic structure of the norovirus protease has been resolved (Nakamura et al., 2005). A two-domain structure was identified as being similar to other viral cysteine proteases, like those of picornavirus 3C proteases that show a chymotrypsin-like fold with a cysteine as the active-site nucleophile (Nakamura et al., 2005; Zeitler et al., 2006).

Similarly to what happens with the picornavirus 3C protease, the scissile bonds of the calicivirus proteases are restricted to a few number of dipeptides, revealing a very high specificity of substrate recognition in the cleavage sites of these enzymes which have also shown to be highly conserved among all caliciviruses (Sosnovtsev, 2010). Hence, the development of substrate-like peptides that block the protease active site constitutes an interesting strategy to inhibit the protease of norovirus. The inhibitors of the 3C protease of picornavirus, such as ruprintrivir, may potentially inhibit the norovirus protease given the overall similarity between the proteases of these viruses. (Binford et al., 2005; De Palma et al., 2008c; Leyssen et al., 2008).

The detailed structural information available for norovirus protease constitutes a good basis for the rational design of protease inhibitors and a valuable tool for the *in silico* screening of large compound libraries (Tan & Jiang, 2008). The recent development of functional assays for the detection of protease activity of calicivirus, using fluorogenic substrate peptides (Zeitler et al., 2006) or bioluminescence technologies (Oka et al., 2011) can constitute useful tools for the screening of protease inhibitors.

However, the role of norovirus protease appears to be more extensive. An association of the protease of MNV with mitochondria was suggested since they were found to co-localize within the cell (Hyde & Mackenzie, 2010). In this case, the protease could be implicated in an up-regulation of apoptosis of norovirus, which is known to occur via a mitochondrial-mediated apoptotic pathway involving caspase-9 (Bok et al., 2009). The involvement of viral protease with apoptotic pathways has been described for their picornavirus counterparts (Drahos & Racaniello, 2009). An association of proteases with mitochondria was linked, in hepatitis A and C viruses, to a cleavage of the mitochondrial antiviral signaling protein (MAVS) (Yang et al., 2007). Since MAVS induces antiviral responses of interferon and NF-kB, one may implicate viral proteases in prevention of immune signaling (Hyde & Mackenzie, 2010).

Whether the protease of MNV plays a role in prevention of immune signaling through the cleavage of MAVS is currently under investigation (Hyde & Mackenzie, 2010). It is known so far that the innate immune response against MNV starts with the recognition of viral RNA by MDA-5 (McCartney et al., 2008), the cytosolic receptor which initiates the antiviral signaling cascade by interacting with MAVS, its downstream partner.

In conclusion, targeting protease may be one of the most promising approaches to inhibit norovirus replication given the important function of this protein.

2.4.6 RNA-dependent RNA polymerase

The RdRp or NS7 is the central enzyme of replication, being responsible for the synthesis of the genomic, subgenomic and antigenomic RNA of norovirus. Given its critical role in norovirus replication, the RdRp constitutes one of the most important antiviral targets.

The crystal structure of the norovirus RdRp in an enzymatically active form has been resolved and overall showed catalytic and structural elements characteristic of RdRp of other positive stranded RNA viruses (Ferrer-Orta et al., 2006; Nakamura et al., 2005; Ng et al., 2004). However, its carboxyl terminus folds into the active site cleft of the enzyme which constitutes a distinctive aspect of the norovirus RdRp deserving special attention when designing an RdRp inhibitor (Hardy, 2005; Ng et al., 2004). It would be expected that a compound that could interrupt the interaction between the carboxyl-terminus and the active site cleft of the RdRp would hamper norovirus replication (Tan & Jiang, 2008).

A more accurate picture of how the RdRp of Norwalk virus functions was provided recently when the crystal structure of the Norwalk virus polymerase was resolved bound to the RNA primer template and to its natural substrate, a nucleoside triphosphate (NTP) (Zamyatkin et al., 2008). This ternary RdRp·RNA·NTP complex revealed details underlying the nucleotidyl transfer reaction, which is thought to be highly conserved among viral polymerases. This complex was also resolved bound to a potent inhibitor of picornavirus polymerases, the 5-nitrocytidine triphosphate (NCT), revealing differences between NCT and NTP complexes, which indicate a novel mechanism of inhibition of the RdRp that should be explored in the design of anti-norovirus compounds mimicking natural nucleosides and nucleotides (Zamyatkin et al., 2008).

A functional assay was developed for the detection of norovirus RdRp activity (Rohayem et al., 2006a; Rohayem et al., 2006b) and the inhibitory activity of some nucleoside analogs such as 2′-arauridine-5′-triphosphate and 3′-deoxyuridine-5′-triphosphate has been reported (Rohayem et al., 2010). Nucleoside analogs display similar mechanisms of inhibition of RdRps and some, like 2′-*C*-methylcytidine, 2'-*C*-methyladenosine and 4′-azidocytidine have shown a broad spectrum of activity against plus stranded RNA viruses, such as picornavirus and HCV (De Palma et al., 2008b; Goris et al., 2007; Klumpp et al., 2006). Hence, this class of compounds has great potential to be inhibitors of the norovirus RdRp and ought to be studied as antiviral drugs.

Ribavirin is a guanosine analogue with broad-spectrum activity against RNA virus. Although it was discovered over 30 years ago, its mechanism of action still remains controversial. Ribavirin was formally approved to treat chronic HCV infections in combination with pegylated interferon and in aerosol form to treat pediatric respiratory

syncytial virus (RSV) infections (Graci & Cameron, 2006). It has also been used against a number of other viruses such as Lassa fever virus, Crimean Congo hemorrhagic fever, hantaviruses and severe acute respiratory syndrome coronavirus (SARS-CoV) (Haagmans & Osterhaus, 2006; Leyssen et al., 2008). Ribavirin has shown to be active against norovirus in the Norwalk replicon model and the MNV model (Chang & George, 2007). The main mechanism of action in the Norwalk replicon model was associated with the depletion of GTP in the cells since the addition of guanosine moderately reversed the antiviral effect of ribavirin (Chang & George, 2007). This depletion of intracellular GTP could be due to the inhibition of the cellular inosine monophosphate dehydrogenase (IMPDH) by ribavirin (Graci & Cameron, 2006; Leyssen et al., 2005). Mycophenolic acid (MPA), a potent noncompetitive inhibitor of IMPDH, also showed to inhibit Norwalk virus replication (Chang & George, 2007).

Another of the proposed mechanisms of ribavirin is an increased mutation frequency via incorporation of ribavirin into newly synthesized genomes leading to error catastrophe (Graci & Cameron, 2006). However, ribavirin seemed not to induce catastrophic mutations in norovirus since there was no increase in the mutation rates in the ribavirin-treated Norwalk replicon-bearing cells (Chang & George, 2007).

Overall, this data indicates that ribavirin or its analogs, such as EICAR (5-ethynyl-1-β-D-ribofuranosylimidazole-4-carboxamide) and viramidine, could constitute a promising starting point in the development of inhibitors of norovirus replication.

2.5 Targeting the norovirus genome

The norovirus RNA genome or viral transcripts also constitute an important target to inhibit the replication of norovirus. Antisense oligonucleotides and siRNAs (small interfering RNA) can be designed to target conserved regions of the norovirus genome with the aim of disrupting viral replication. For that, these oligonucleotides need to fulfill some requisites, namely the target sequence has to be involved in viral replication, accessible for oligonucleotide hybridization and conserved among different viral strains (Spurgers et al., 2008).

The existence of conserved secondary structures among calicivirus genomes opens the possibility of designing an oligonucleotide that would present a broad spectrum activity among noroviruses or troughout the family *Caliciviridae*. These conserved structures include 5′ terminal stem-loops, 3′ terminal hairpins, a stem-loop just upstream of the ORF1/2 junction in the antigenomic strand and a stem-loop at the 5′ end of the polymerase coding region with a motif characteristic of picornavirus *cis*-acting replication elements (*cre* elements) that dictate VPg uridylylation (Simmonds et al., 2008; Victoria et al., 2009). Some of these structures were found to be critical for the replication of MNV and for its infectivity (Simmonds et al., 2008). By using MNV reverse genetics system it was demonstrated that the disruption of the 5′-stem loops or the 3′-hairpins strongly impaired MNV replication *in vitro* (Simmonds et al., 2008). Moreover, a polypyrimidine tract located at the 3′-end of the genome has been incriminated in regulating viral fitness and virulence of MNV *in vivo* (Bailey et al., 2010). The important roles played by these conserved RNA structures in norovirus replication and virulence makes them potentially good antiviral targets.

The 5′-UTR (untranslated region) sequence of the norovirus genome has already been successfully targeted by an antisense strategy. A panel of peptide-conjugated phosphorodiamidiate morpholino oligomers (PPMOs) specific for the 5′-UTR of MNV proved to be effective in inhibiting its replication *in vitro* (Bok et al., 2008). Also, a consensus PPMO (designated *Noro 1.1*), designed to target the corresponding region of several diverse human norovirus genotypes, inhibited Norwalk virus protein expression in replicon-bearing cells (Bok et al., 2008). Moreover, PMOs targeting the 5′-UTR of the FCV genome were used successfully in three clinical trials during FCV outbreaks in kittens (Smith et al., 2008). Overall, these studies suggests that PPMOs directed against the relatively conserved 5′-end of the norovirus genome may show broad antiviral activity against this genetically diverse group of viruses and might translate into a successful clinical application but only further studies in animal models will say.

An alternative nucleic acid-based strategy is the use of RNA interference (RNAi) for silencing the viral genome. A wide range of viruses have been inhibited with RNAi (Leonard & Schaffer, 2006) and concerning calicivirus the first preliminary results have been published (Bergmann & Rohayem, 2010; Rohayem et al., 2010). In this study, siRNAs targeting the 5′-UTR and the subgenomic region of the FCV genome were successful in inhibiting FCV replication *in vitro*, namely by reducing infectivity, reducing the levels of viral genomic RNA and inhibiting viral translation.

2.6 Other strategies to stop norovirus replication

The enhancement of the host cell's antiviral mechanisms may constitute a valuable strategy to inhibit norovirus replication. Interferons (IFNs) are critical components of the innate immune response which establish an antiviral state of cells through interactions with IFN receptors expressed in nearly all nucleated cells. The binding of IFNs to their receptors triggers activation of STATs (signal transducer and activator of transcription) and cascade events which results in the induction of various antiviral proteins, such as RNA-dependent protein kinase (PKR) and RNase L (Samuel, 2001). However, many viruses are armed with anti-IFN mechanisms, such as those counteracting the STATs and inhibiting IFN synthesis (Samuel, 2001).

Interferons, both type I (IFN-α) and type II (IFN-γ), showed to inhibit the replication of norovirus in the Norwalk replicon model (Chang & George, 2007; Chang et al., 2006). Norwalk virus did not present a strong anti-IFN mechanism in the replicon-bearing cells, and this may be a reason for its high sensitivity to IFNs (Chang & George, 2007; Chang et al., 2006). It was also demonstrated that IFN responses were critical to control MNV infection *in vivo* and inhibited viral replication *in vitro* (Karst et al., 2003; Wobus et al., 2004). Both type I and type II IFNs block the translation of viral proteins of MNV but while type II IFN-mediated inhibition is dependent on the well-characterized interferon-induced antiviral molecule PKR, type I IFN-mediated inhibition occur through a PKR-independent process (Changotra et al., 2009). This data suggests that IFN may be a good therapeutic option for norovirus gastroenteritis.

The cellular pathway of cholesterol biosynthesis has been shown to be upregulated during the replication of several viruses, including HIV and HCV (Giguere & Tremblay, 2004; Ye, 2007). A study using Norwalk replicon system demonstrated that cholesterol pathways were also important in the replication of norovirus (Chang, 2009). Statins, such as

simvastatin and lovastatin, are well known drugs that interfere with cholesterol pathways by inhibiting *de novo* synthesis of cholesterol through inhibition of HMG-CoA (3-hydroxy-3-methyl glutaryl-coenzyme A), reducing plasma cholesterol levels by upregulating low density lipoprotein receptor (LDLR) and promoting the uptake of LDL bound cholesterol to cells. It has been demonstrated that the use of statins resulted in a reduction of HCV replication through blockage of protein geranylgeranylation and the proper formation of viral replicase complexes (Ye, 2007; Ye et al., 2003). On the contrary, the inhibition of cholesterol biosynthesis using statins significantly enhanced the replication of Norwalk virus which was correlated with an increased expression of LDLR (Chang, 2009). It was postulated that LDLR could play an important direct role in virus replication such as participating in viral replication complexes as an essential cofactor (Chang, 2009).

The activity of acyl-CoA:cholesterol acyltransferase (ACAT) is also an important factor for cholesterol biosynthesis. Unlike statins, treatment with ACAT inhibitors such as Cl-976, Sandoz 58-035, YIC-C8-434 and pyripyropene resulted in reduced levels of Norwalk replication and interestingly, in reduced levels of LDLR (Chang, 2009). This data indicate that ACAT may be a novel target for inhibiting norovirus replication and its inhibitors could be further developed as anti-norovirus drugs.

3. Final remarks

Antiviral therapy is still not available today for norovirus and certainly a long road still lies ahead. A significant progress has been made in the elucidation of the replication strategy of norovirus, for which the use of surrogate viruses, the generation of a Norwalk replicon model, the available crystal structures of norovirus proteins were landmark developments that helped this giant pursuit.

In this chapter, we review and speculate about potential targets and antiviral strategies. Many were the targets suggested, however we believe that priority should be given to viral enzymes of replication, such as the RdRp and protease. Besides being key enzymes in norovirus life cycle, they are conserved across this genetically diverse group of viruses and divergent enough from cellular enzymes for their inhibitors to have good selectivity and minimal toxicity. Moreover, viral enzymes of replication are in general less prone to variation than structural proteins, minimizing the emergence of drug resistance.

As the understanding of norovirus replication deepens, one could look forward to new opportunities for the development of innovative antiviral strategies targeting this important human pathogen in a near future.

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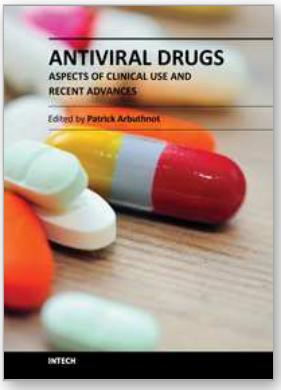

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The articles that appear in Antiviral Drugs - Aspects of Clinical Use and Recent Advances cover several topics that reflect the varied mechanisms of viral disease pathogenesis and treatment. Clinical management and new developments in the treatment of virus-related diseases are the two main sections of the book. The first part reviews the treatment of hepatitis C virus infection, the management of virus-related acute retinal necrosis, the use of leflunomide therapy in renal transplant patients, and mathematical modeling of HIV-1 treatment responses. Basic research topics are dealt with in the second half of the book. New developments in the treatment of the influenza virus, the use of animal models for HIV-1 drug development, the use of single chain camelid antibodies against negative strand RNA viruses, countering norovirus infection, and the use of plant extracts to treat herpes simplex virus infection are described. The content of the book is not intended to be comprehensive, but aims to provide the reader with insights into selected aspects of established and new viral therapies.

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