Spring viraemia of carp virus: recent advances

Usama Ashraf,1,2 Yuyan Lu,4 Li Lin,1,2 Junfa Yuan,1,2 Min Wang1,2 and Xueqin Liu1,2

1Department of Aquatic Animal Medicine, College of Fisheries, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China
2Freshwater Aquaculture Collaborative Innovation Center of Hubei Province, Wuhan 430070, PR China
3State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan, Hubei 430070, PR China
4Department of Public Health Sciences, University of Hawaii, Manoa, HI 96822, USA

Spring viraemia of carp is an environmentally and economically important disease affecting cyprinids, primarily common carp (Cyprinus carpio). The causative agent of this disease is Spring viraemia of carp virus (SVCV) – a member of the genus Vesiculovirus of the family Rhabdoviridae. The disease is presently endemic in Europe, America and several Asian countries, where it causes significant morbidity and mortality in affected fish. SVCV infection is generally associated with exophthalmia; abdominal distension; petechial haemorrhage of the skin, gills and internal organs; degeneration of the gill lamellae; a swollen and coarse-textured spleen; hepatic necrosis; enteritis; and pericarditis. The SVCV genome is composed of linear, negative-sense, ssRNA containing five genes in the order 3’-N–P–M–G–L-5’, encoding a nucleoprotein, phosphoprotein, matrix protein, glycoprotein and RNA-dependent RNA polymerase, respectively. Fully sequenced SVCV strains exhibit distinct amino acid substitutions at unique positions, which may contribute to as-yet unknown strain-specific characteristics. To advance the study of SVCV and the control of spring viraemia of carp disease in the future, this review summarizes our current understanding of SVCV in terms of its genomic characteristics, genetic diversity and pathogenesis, and provides insights into antiviral immunity against SVCV, diagnosis of SVCV and vaccination strategies to combat SVCV.

Introduction

Spring viraemia of carp virus (SVCV) is a member of the genus Vesiculovirus of the family Rhabdoviridae (Fijan, 1999; Ahne et al., 2002) and is responsible for the highly contagious spring viraemia of carp disease associated with haemorrhagic symptoms in cyprinids, especially common carp (Cyprinus carpio) (Fijan, 1984, 1999; Baudouy et al., 1980; Ahne et al., 2002). Natural SVCV infections have been reported in other cyprinid fish, including goldfish (Carassius auratus), koi (Cyprinus carpio koi), silver carp (Hypophthalmichthys molitrix), crucian carp (Carassius carassius), bighead carp (Aristichthys nobilis), grass carp (Ctenopharyngodon idella), tench (Tinca tinca) and orfe (Leuciscus idus) (Fijan, 1984; Shchelkunov & Shchelkunova, 1989; Ahne et al., 2002). Experimental infections have been reported in other cyprinid fish, including goldfish (Carassius auratus), koi (Cyprinus carpio koi), silver carp (Hypophthalmichthys molitrix), crucian carp (Carassius carassius), bighead carp (Aristichthys nobilis), grass carp (Ctenopharyngodon idella), tench (Tinca tinca) and orfe (Leuciscus idus) (Fijan, 1984; Shchelkunov & Shchelkunova, 1989; Ahne et al., 2002). Experimental infections have been reported in zebrafish (Danio rerio), golden shiner (Notemigonus crysoleucas), roach (Rutilus rutilus), guppy (Lebistes reticulatus), pumpkinseed (Lepomis gibbosus), northern pike (Esox lucius), fathead minnow (Pimephales promelas), emerald shiner (Notropis atherinoides) and white sucker (Catostomus commersonii) (Ahne, 1985; Haenem & Davidse, 1993; Ahne et al., 2002; Misk et al., 2015). SVCV infection is highly lethal in young fish, with mortality rates up to 90% (Baudouy et al., 1980), and thus causes substantial economic losses to the aquaculture industry.

SVCV was first detected in Yugoslavia in 1971 (Fijan, 1972), and was subsequently reported in the Americas (Miller et al., 2007; Warg et al., 2007) and Asia (Teng et al., 2007). SVCV infection has now been reported in many European countries, including the UK, Denmark, Germany, the Netherlands, Austria, Spain, France, the Czech Republic and several western states of the former USSR (Russia, Georgia, Belarus, Moldova, Ukraine and Lithuania) (Marcotegui et al., 1992; Stone et al., 2003; Hoffmann et al., 2005; Shchelkunov et al., 2005; Miller et al., 2007; Basic et al., 2009). Outbreaks of spring viraemia of carp usually occur in the spring, when the water temperature begins to rise after a cold winter (Ahne, 1986). Studies in carp have shown that few adult fish are infected when the water temperature is > 17 °C, but juveniles can be infected even at 22–23 °C (Ahne, 1986). Other risk factors associated with morbidity and mortality include fish
density, geographical location, fish and the immune status of susceptible fish (Ahne, 1986). SVCV infection can be spread by fomites and parasitic invertebrates. It is difficult to eradicate the virus from affected ponds once the infection is established and elimination of the virus may require destruction of all aquatic life (Fijan, 1984; Ahne et al., 2002). The expansion of the geographical and host range of SVCV thus poses a major threat not only to the seafood and ornamental fish trade industries, but also to endemic wild fish populations (Ahne et al., 2002). Due to the highly infectious nature of SVCV and its potential impact on susceptible fish populations globally, any detection of SVCV requires notification within 48 h to the International Office of Epizootics. SVCV is one of nine piscine viruses recognized by the International Office of Epizootics as a notable animal disease.

In recent years, a number of studies have explored SVCV-induced antiviral and immune evasion mechanisms. New vaccination strategies and diagnostic assays have also been implemented to combat SVCV. This review sheds light on the latest findings about SVCV, including genetic diversity amongst SVCV strains, pathogenesis, antiviral immunity, and recent advances in diagnosis and vaccine development.

### Genome structure and characteristics

SVCV exhibits a bullet-shaped morphology, and measures ~80–180 nm in length and 60–90 nm in diameter (Ahne et al., 2002). It has a negative-sense, ssRNA genome of ~11 kb. It contains five ORFs encoding five structural proteins: a nucleoprotein (N), phosphoprotein (P), matrix protein (M), glycoprotein (G) and RNA-dependent RNA polymerase (L) (Hoffmann et al., 2002; Teng et al., 2007; Zhang & Gui, 2015). The five viral genes are organized in the order typical of rhabdoviruses: 3’-N–P–M–G–L-5’ (Hoffmann et al., 2002; Teng et al., 2007; Zhang & Gui, 2015). The SVCV model structure and genome organization are shown in Fig. 1. Characteristics of all five ORFs, including total length, encoded protein, and lengths of 3' and 5' UTRs are summarized in Table 1.

The L protein is involved in transcription and viral replication, which are achieved through interaction of the L protein with the N and P proteins (Roy & Clewley, 1978). The G protein forms the trimeric spikes or peplo-mers on the virus outer surface and helps the virus to induce receptor-mediated endocytosis (Hill et al., 1975; Bishop & Smith, 1977). The surface G protein is the most important viral antigenic protein that determines the infectivity and serological properties of the virus (Hill et al., 1975; Bishop & Smith, 1977; Johnson et al., 1999). The N protein is a highly abundant viral protein that, in association with viral RNA, gives a helical symmetry to the nucleocapsid (Sokol & Koprowski, 1975). The P protein is a component of the nucleocapsid that interacts with N and L proteins to mediate transcription (Roy, 1981). Similar to other vesiculoviruses, SVCV has one type of M protein that provides the bullet-shaped structure of the virus and binds the nucleocapsid to the cytoplasmic domains of the G proteins embedded in the viral envelope (Kiuchi & Roy, 1984).

The SVCV genome contains a putative leader region of 59 bases at the 3’ terminal region, followed by a consensus start signal sequence (AACAG; anti-genomic orientation) for initiation of transcription of the N gene (Hoffmann et al., 2002; Warg et al., 2007). The four SVCV genome junctions (N–P, P–M, M–G and G–L) are evolutionarily conserved, with a polyadenylation or transcription stop signal sequence [TATG(A)7; anti-genomic orientation] at the end of each gene (Hoffmann et al., 2002; Warg et al., 2007). The untranscribed N–P, P–M and M–G intergenic regions all have the single dinucleotide CT, whereas the G–L intergenic region has the tetranucleotide CTAT (Hoffmann et al., 2002; Warg et al., 2007). Compared with other rhabdoviruses, the SVCV intergenic regions and regulatory signal sequences exhibit complete sequence homology to all vesiculoviruses, but are different from lyssaviruses and novirhabdoviruses (Hoffmann et al., 2002; Warg et al., 2007). The SVCV trailer region contains polyadenylation signal sequences for the L gene (Hoffmann et al., 2002; Warg et al., 2007).

### Genetic diversity amongst SVCV strains

Many SVCV strains have been identified. As in other RNA viruses that can evolve rapidly, a high level of plasticity has been reported in the SVCV genome. Broadly, SVCV isolates are divided into two clades: an Asian clade and a European clade (Stone et al., 2007; Miller et al., 2003). Based on nucleotide sequence analysis of the G gene, SVCV isolates are further classified into four genogroups: Ia, Ib, Ic and Id (Warg et al., 2007; Zhang et al., 2009). Genogroup Ia contains isolates from Asia, the UK and the Americas; Ib and Ic contain isolates from Eastern Europe; and Id contains isolates from the UK and some other European countries (Stone et al., 2003; Hoffmann et al., 2005; Miller et al., 2007; Zhang et al., 2009; Padhi & Verghese, 2012). The genetic clustering of SVCV isolates is closely associated with geographical location, suggesting that the virus has evolved independently in different geographical regions (Stone et al., 2013). In addition, different isolates have evolved at varied rates, with the Ia group evolving the most quickly (Padhi & Verghese, 2012). The nucleotide substitution rates in the P and G genes of genogroup Ia are ~3.5 times higher than in the same genes of the Id group (Padhi & Verghese, 2012). Compared with the N and G genes, the P gene exhibits a higher degree of genetic variation and is considered to be a useful marker for studying SVCV epidemiology (Sokol & Koprowski, 1975; Miller et al., 2007).
The genomes of most identified SVCV strains have been partially sequenced. To date, only five strains have been completely sequenced: Fijian, Björklund, SVCV-A1, SVCV-C1 and SVCV-265 (Björklund et al., 1996; Hoffmann et al., 2002; Teng et al., 2007; Zhang et al., 2009; Xiao et al., 2014). A comparative analysis of all completely sequenced strains relative to the Fijian strain as a reference strain is summarized in Table 2. These analyses were performed by BLAST analysis (http://www.ncbi.nlm.nih.gov/blast).

Each fully sequenced strain exhibits unique amino acid substitutions that may contribute to as-yet unknown strain-specific characteristics. Moreover, specific amino acid substitutions are observed in all five coded proteins (Fig. 2). Strain-specific substitutions in the N protein are T149A (Björklund); D409V (SVCV-A1); T95I, D201N and D407N (SVCV-C1); and R63K and I182V (SVCV-265). Strain-specific substitutions in the P protein are G204S and R295K (SVCV-C1); and E90K, N101S, K257E, C277R and R282H (SVCV-265). Strain-specific substitutions in the M protein are T10I (Björklund) and S56T (SVCV-265). Strain-specific substitutions in the G protein are L497R (Björklund); D165E, V357I and I364T (SVCV-A1); G31W, G155E, R416Q and T470M (SVCV-C1); and F10L, A260T, S362N and A500T (SVCV-265). Strain-specific substitutions in the L protein are S82G, S673R, Q1424L, G1754D and R1897G (Björklund); Y25L, R26S, S816A, F827Y, L828F, P829A, I1483S and N1650D (SVCV-A1); R56K, H183N, G703D, T1109I, T1396I, T1823M and D1831D (SVCV-C1); and E1288K, V1303I, I1383V, I1402V and L1494I (SVCV-265). Interestingly, SVCV-C1 also exhibits a six-residue insertion (KSLANA) in the L protein (Fig. 2) that is not observed in the other four

### Table 1. Characteristics of the SVCV genome

<table>
<thead>
<tr>
<th>ORF</th>
<th>Protein</th>
<th>Gene length (bp)</th>
<th>ORF length (bp)</th>
<th>3’ UTR length (bp)</th>
<th>ORF start position (nt)</th>
<th>ORF end position (nt)</th>
<th>5’ UTR length (bp)</th>
<th>Protein length (aa)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ORF1</td>
<td>N</td>
<td>1335</td>
<td>1272</td>
<td>10</td>
<td>70</td>
<td>1326</td>
<td>68</td>
<td>418</td>
</tr>
<tr>
<td>ORF2</td>
<td>P</td>
<td>967</td>
<td>930</td>
<td>10</td>
<td>1407</td>
<td>2336</td>
<td>27</td>
<td>309</td>
</tr>
<tr>
<td>ORF3</td>
<td>M</td>
<td>716</td>
<td>672</td>
<td>10</td>
<td>2376</td>
<td>3047</td>
<td>34</td>
<td>223</td>
</tr>
<tr>
<td>ORF4</td>
<td>G</td>
<td>1588</td>
<td>1530</td>
<td>10</td>
<td>3094</td>
<td>4623</td>
<td>48</td>
<td>509</td>
</tr>
<tr>
<td>ORF5</td>
<td>L</td>
<td>6325</td>
<td>6288</td>
<td>10</td>
<td>4686</td>
<td>10973</td>
<td>Trailer</td>
<td>2095</td>
</tr>
</tbody>
</table>

Fig. 1. Schematic of SVCV and its genome. (a) Model of viral structure. (b) Genome organization showing encoded proteins, gene junctions, and leader and trailer regions. The total genome length is ~11 kb. The numbers indicate the start and end positions of the respective ORFs.
strains, providing a unique hallmark for SVCV-C1. The functional importance of the strain-specific amino acid substitutions and insertion is presently unknown. However, these mutations may play roles in the viral infectivity, antigenicity and virulence of these strains. Further experimental studies focusing on pathogenesis will be important to estimate the possible threat of these viral strains to worldwide aquaculture.

Table 2. Nucleotide and amino acid sequence similarity amongst fully sequenced SVCV strains

All strains compared with Fijian as the reference strain.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Fijian</th>
<th>Björklund</th>
<th>SVCV-A1</th>
<th>SVCV-C1</th>
<th>SVCV-265</th>
</tr>
</thead>
<tbody>
<tr>
<td>GenBank accession number</td>
<td>AJ318079</td>
<td>NC_002803</td>
<td>DQ097384</td>
<td>EU177782</td>
<td>KJ513477</td>
</tr>
<tr>
<td>Geographical origin</td>
<td>Europe</td>
<td>Europe</td>
<td>China</td>
<td>China</td>
<td>China</td>
</tr>
<tr>
<td>Genome length (bp)</td>
<td>11019</td>
<td>11019</td>
<td>11100</td>
<td>11047</td>
<td>11029</td>
</tr>
<tr>
<td>N gene similarity (%)</td>
<td>–</td>
<td>99</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>P gene similarity (%)</td>
<td>–</td>
<td>99</td>
<td>90</td>
<td>90</td>
<td>89</td>
</tr>
<tr>
<td>M gene similarity (%)</td>
<td>–</td>
<td>99</td>
<td>94</td>
<td>93</td>
<td>93</td>
</tr>
<tr>
<td>G gene similarity (%)</td>
<td>–</td>
<td>99</td>
<td>92</td>
<td>91</td>
<td>92</td>
</tr>
<tr>
<td>L gene similarity (%)</td>
<td>–</td>
<td>99</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>N protein similarity (%)</td>
<td>–</td>
<td>99</td>
<td>97</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>P protein similarity (%)</td>
<td>–</td>
<td>99</td>
<td>90</td>
<td>89</td>
<td>89</td>
</tr>
<tr>
<td>M protein similarity (%)</td>
<td>–</td>
<td>99</td>
<td>98</td>
<td>98</td>
<td>98</td>
</tr>
<tr>
<td>G protein similarity (%)</td>
<td>–</td>
<td>99</td>
<td>94</td>
<td>94</td>
<td>94</td>
</tr>
<tr>
<td>L protein similarity (%)</td>
<td>–</td>
<td>99</td>
<td>97</td>
<td>97</td>
<td>98</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Protein</th>
<th>Björklund strain</th>
<th>Fijian strain</th>
<th>SVCV-A1</th>
<th>SVCV-C1</th>
<th>SVCV-265</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>VKESLRLHKTVKTMI</td>
<td>YSKEKKKTVK</td>
<td>DSD</td>
<td>DSD</td>
<td>DSD</td>
</tr>
<tr>
<td>P</td>
<td>NVDSNLDNEDKEK</td>
<td>EVKKEK</td>
<td>NDN</td>
<td>NDN</td>
<td>NDN</td>
</tr>
<tr>
<td>M</td>
<td>DQAVNDKTNNDN</td>
<td>DK</td>
<td>NDN</td>
<td>NDN</td>
<td>NDN</td>
</tr>
<tr>
<td>G</td>
<td>VSLNIDINGTCDE</td>
<td>IGG</td>
<td>NDN</td>
<td>NDN</td>
<td>NDN</td>
</tr>
<tr>
<td>L</td>
<td>ATQK</td>
<td>YG</td>
<td>NDN</td>
<td>NDN</td>
<td>NDN</td>
</tr>
</tbody>
</table>

Fig. 2. Comparison of protein sequences from fully sequenced SVCV strains. N protein (aa 1–418 amino acids), P protein (aa 1–309), M protein (aa 1–223), G protein (aa 1–509) and L protein (aa 1–2095). Fijian is the reference strain. Amino acid substitutions are shown in bold.
The phylogeny of all sequenced SVCV strains based on their complete genomes and on the analysis of individual protein sequence is shown in Figs 3 and 4, respectively. The phylogenetic trees are reconstructed by the neighbour-joining method using MEGA (Tamura et al., 2011).

Reverse genetics is a powerful tool to study gene functions of RNA viruses. A method of recovering negative-strand RNA viruses from the full-length cDNA clones was first developed for rabies virus (Schnell et al., 1994). This method involves the expression of a full-length positive-strand (antigenomic) RNA of the virus genome by vaccinia-driven T7 RNA polymerase (Fuerst et al., 1994; Schnell et al., 1986). After this recovery, many negative-strand RNA viruses have been recovered using similar technique, including human respiratory syncytial virus (Collins et al., 1995), vesicular stomatitis virus (Lawson et al., 1995) and infectious hematopoietic necrosis virus (IHNV) (Biacchessi et al., 2000) and viral hemorrhagic septicemia virus (VHSV) (Ammayappan et al., 2011). However, no efforts have been continued for the development of reverse genetics system for SVCV. The development of this technique could be a major breakthrough in the field of SVCV research, and may pave the way to basic studies of gene function and viral pathogenesis.

**Clinical signs, gross lesions and histopathology of SVC**

SVCV infection is generally associated with non-specific symptoms, such as exophthalmia, abdominal distension and oedema of the vent region (Ahne et al., 2002). Affected fish often have darkened bodies and pale gills. Petechial haemorrhages can be seen on the skin, gills, eyes and internal organs, particularly on the walls of the swim bladder (Ahne et al., 2002; Sanders et al., 2003). Other lesions may include degeneration of the gill lamellae, oedematous internal organs, swollen and coarse-textured spleen, hepatic necrosis, enteritis, and pericarditis (Ghasemi et al., 2014; Misk et al., 2015).

There are only a few reports on histological changes of SVCV-infected fish (Negele, 1977; Misk et al., 2015). The histological changes associated with liver may range from perivasculitis to panvasculitis with a higher degree of oedematization and loss of structure of blood vessels walls (Negele, 1977). The liver parenchyma can show hyperaemia, multifocal necrosis and adipose degeneration (Ghasemi et al., 2014), whilst the spleen is often hyperaemic, showing a considerable hyperplasia of the reticuloendothelium (Negele, 1977). Siderocytes and cells with increased lipofuscin storage may be present. Lymph vessels can be extremely dilated and engorged with detritus, macrophages and lymphocytes (Ahne et al., 2002). Multifocal necrosis and non-purulent inflammation often occur in the pancreas of affected fish, and the heart shows pericarditis and discontinuous myocardial degeneration (Negele, 1977; Misk et al., 2015). The visceral and parietal serosa of the peritoneum show peritonitis. In the intestine, perivasculitis with subsequent atrophy of the villi is often observed. The renal tubuli can be obstructed by the tube casts, and may exhibit vacuolation and hyaline degeneration (Ahne et al., 2002; Misk et al., 2015). The epithelial layer of the swim bladder changes into a discontinuous multilayer and haemorrhages are commonly observed in the submucosa (Ahne et al., 2002).

**Pathogenesis of SVCV**

Currently, little is known about the pathogenic mechanisms used by SVCV to modulate the host immune response and only a few studies have provided valuable insights into the molecular aspects of SVCV pathogenesis. One pathogenic mechanism used by SVCV is the induction of autophagy (Liu et al., 2015). Autophagy is a cellular pathway that has important roles in viral infection and pathogenesis (Orvedahl & Levine, 2008). The outcomes of virally induced autophagy can be very different depending on viral type. Viruses such as dengue, polio and coxsackievirus utilize autophagy pathways to facilitate their own survival (Kirkegaard et al., 2004; Jackson et al., 2005; Wong et al., 2008). In contrast, autophagy limits the replication of VHSV (Garcia-Valtanen et al., 2014), Sendai virus (Shoji-Kawata & Levine, 2009), Sindbis virus (Liang et al., 1998) and herpes simplex virus I (Santana et al., 2012) via degradation of viral proteins. SVCV falls into
the first category, using the autophagy pathway to facilitate its own replication through a process induced by the SVCV G protein (Liu et al., 2015). Autophagy enhances the survival of SVCV-infected cultured cells by eliminating damaged mitochondrial DNA generated during viral infection. Furthermore, SVCV induces autophagy by modulating the extracellular signal-regulated kinase/mammalian target of rapamycin (ERK/mTOR) signalling pathway. More precisely, SVCV triggers expression of ERK1/2 protein to suppress the activity of mTOR protein (Liu et al., 2015). These findings identify potential targets or pathways to block SVCV infection using respective drugs.

Immune signalling pathways are regulated by multiple cytokines, including IFNs and ILs. Although cytokines are required to control infection, their overexpression can lead to local or systemic pathological changes (Van Reeth, 2000; Julkunen et al., 2001). Suppressor of cytokine signalling 3 (SOCS3) acts as a negative regulator of cytokine receptor signalling (Yoshimura et al., 2007). Notably, overexpression of SOCS3 can inhibit IFN-β/γ and IL-6 signalling in humans with high specificity and potency (Pothlichet et al., 2008; Wang & Secombes, 2008; Babon et al., 2014). SOCS3 is significantly upregulated in immune organs of SVCV-infected carp, indicating its role in modulating the viral-induced immune response and in preventing overactivation of some cytokines (Xiao et al., 2010). Together, these data suggest the potential role of SOCS3 in enhancing SVCV replication.

Haem oxygenase 1 (HO-1), also known as heat-shock protein 32, is a cytoprotective enzyme associated with protection of the body against oxidative stress during inflammatory processes (Otterbein & Choi, 2000). HO-1 exhibits antiviral properties against multiple viruses, including hepatitis B (Qiu et al., 2010), hepatitis C (Zhu et al., 2008; Lehmann et al., 2010) and human immunodeficiency virus (Devadas & Dhawan, 2006). In contrast, viral infection can suppress expression of HO-1, perhaps contributing to viral pathogenesis (Marinissen et al., 2006). HO-1

---

**Fig. 4.** Phylogenetic trees based on amino acid sequence analysis of (a) N, (b) P, (c) M, (d) G and (e) L proteins. This figure illustrates the close genetic relationship between completely sequenced SVCV strains based on N, P, M, G, and L protein sequence analysis. The number at each node is the percentage of 1000 bootstrap replicates. Fijian: N (CAC51333), P (CAC51334), M (CAC51335), G (CAC51336) and L (CAC51337). Björklund: N (NP_116744), P (NP_116745), M (NP_116746), G (NP_116747) and L (NP_116748). SVCV-A1: N (AAZ20272), P (ABD67435), M (AAZ20271), G (AAZ20272) and L (ABW24036). SVCV-C1: N (ABW24033), P (ABW24034), M (ABW24035), G (ABW24036) and L (ABW24037). SVCV-265: N (AIU34414), P (AIU34415), M (AIU34416), G (AIU34417) and L (AIU34418). Bars, substitutions per site. (GenBank accession numbers are given in parentheses.)
Antiviral immunity against SVCV

Recognition of viral elements by host pattern recognition receptors is essential for initiation of an appropriate innate antiviral immune response and subsequent activation of an adaptive immune response. Host recognition of viral molecular motifs triggers antiviral signalling pathways that lead to production of type I IFN, IFN-induced genes and inflammatory cytokines, thereby restricting viral propagation (Honda et al., 2005; Melchjorsen et al., 2010). Mammalian virus-sensing pattern recognition receptors include Toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors, retinoic acid-inducible gene 1 (RIG-I)-like receptors (RLRs), HIN-200 family receptors and a number of intracellular DNA receptors (Roberts et al., 2009; O'Neill & Bowie, 2010; Unterholzner, 2013). RLRs recognize intracellular viral RNAs and consist of three members: RIG-I, melanoma differentiation-associated gene 5 (MDA5), and laboratory of genetics and physiology 2 (LGP2) (Yoneyama et al., 2005; Bruns & Horvath, 2012). RLRs recognize viral RNAs through the DExD/H box RNA helicase domain, and trigger activation of downstream signalling through the N-terminal caspase activation and recruitment domain (CARD) (Seth et al., 2005; O'Neill & Bowie, 2010). The adaptor protein that associates RLRs with downstream signalling molecules is the mitochondrial antiviral signalling (MAVS) protein, also known as virus-induced signalling adaptor (VISA), IFN-β promoter stimulator 1 (IPS-1) and CARD adaptor inducing IFN-β (Cardif) (Kawai et al., 2005; Meylan et al., 2005; Seth et al., 2005; Xu et al., 2005).

Orthologues of human RLRs have been characterized in teleost fish, including grass carp (Chen et al., 2012), rainbow trout (Chang et al., 2011), common carp (Feng et al., 2011) and channel catfish (Rajendran et al., 2012). Fish RIG-I recognizes RNA viruses such as grass carp reovirus (Chen et al., 2012) and VHSV (Biachesi et al., 2009). Zou et al. (2015) investigated an induced antiviral immune response of RIG-I through augmentation of RIG-I/MAVS-mediated signalling in zebrafish upon infection with SVCV (Zou et al., 2015). Zebrafish has two variants of RIG-I: an insertion variant with 38 amino acids inserted in the N-terminal CARD2 domain (RIG-1a) and a typical variant (RIG-1b) (Zou et al., 2015). After SVCV infection, both variants become more highly expressed (Zou et al., 2015), suggesting that RIG-I may have a potent role in recognition of negative-sense ssRNA viruses. Overexpression of RIG-Ib in cultured zebrafish embryonic fibroblast cells leads to inhibition of SVCV through induction of the type I IFN response, whereas overexpression of RIG-Ia has no significant effect on IFN activation or anti-SVCV activity (Zou et al., 2015). Moreover, both RIG-Ia and RIG-Ib are linked with the downstream adaptor protein MAVS (Zou et al., 2015). Co-transfection of RIG-Ia with RIG-Ib or MAVS resulted in enhanced type I IFN promoter activity and upregulation of both IFN regulatory factor 3 (IRF3) and myxovirus resistance (Mx) gene, implying that RIG-Ia has a role as an enhancer in the RIG-Ib/MAVS-mediated signalling pathway (Zou et al., 2015).

Three MAVS variants (MAVS-1a, -1b and -1c) have been isolated from human cells (Lad et al., 2008). Variants 1a and 1b, which have uncharacterized sequences, have opposite effects on IFN induction (Lad et al., 2008). In contrast, variant 1c, which lacks a C-terminal transmembrane domain, has no influence on either IRF3 or NfκB signalling pathways (Lad et al., 2008). MAVS orthologues have been identified in zebrafish (Xiong et al., 2012), salmon (Lauksund et al., 2009), Japanese flounder (Simora et al., 2010), spotted green pufferfish (Xiang et al., 2011) and grass carp (Wan et al., 2013). Although alternative splicing variants of MAVS have been reported in zebrafish and salmon (Biachesi et al., 2009), their functions and mechanisms of interacting with other signalling proteins remain poorly understood. In zebrafish, Chen et al. (2015b) characterized two MAVS variants: MAVS-tv1 and MAVS-tv2. MAVS-tv1 is a full-length form of MAVS, whereas MAVS-tv2 is a truncated variant without a C-terminal transmembrane domain (Chen et al., 2015b). Overexpression of MAVS-tv1 or -tv2 in zebrafish embryonic fibroblast cells results in enhanced transcription of antiviral genes such as those for IFN1, IFN2, IFN3, IFN4, Mxa, Mxb, Mxc and radical S-adenosyl methionine domain containing protein 2 (RSAD2), and thereby confers an antiviral state against SVCV (Chen et al., 2015b). Furthermore, MAVS-tv1 interacts with RIG-I in a positive feedback mechanism to enhance the accumulation of RIG-I transcripts, whereas MAVS-tv2 cooperates with MDA5 to enhance accumulation of MAVS-tv2 transcripts (Chen et al., 2015b). These findings suggest a role for fish MAVS in inducing antiviral immunity against SVCV.
RIG-I, MAVS, TNF receptor-associated factor 3 (TRAF3) and TANK-binding kinase 1 (TBK1) are four pivotal molecules in the RIG-I-mediated antiviral signalling cascade. Upon SVCV infection in carp, the expression of each of these molecules is significantly elevated in spleen, intestine and head kidney tissues (Feng et al., 2011). In addition, carp IRF3 and IRF7 are also upregulated in these same tissues following SVCV infection (Feng et al., 2011), indicating that carp RIG-I and MAVS may be involved in antiviral responses through RIG-I-mediated viral recognition signalling pathways in a TRAF3/TBK1-dependent manner. Furthermore, the expression of TLR9, IRF4, and other antiviral genes such as those for virus-induced gene 1 (VIG-1), daf-12 redundant function 1 (DreI), protein kinase RNA-activated (PKR) and adenosine deaminase, RNA-specific (Adar) are also modulated (Adamek et al., 2012), although the exact mechanisms by which these proteins are regulated remain unclear. A comprehensive view of host innate anti-SVCV mechanisms is shown in Fig. 5.

NK-lysins are antimicrobial cationic proteins produced by cytotoxic T-lymphocytes and NK-cells. They are stored in cytoplasmic granules and released upon target cell recognition via MHC class I to induce apoptosis in the infected cells (Trapani & Smyth, 2002). The inhibitory role of NK-lysins against bacterial, fungal and parasitic infections has been well established (Wang et al., 2000; Jacobs et al., 2003; Hirono et al., 2007), but their role in protection against viral infections is relatively unknown. Recently, NK-lysin genes were characterized in zebrafish and their expression was investigated in SVCV-infected zebrafish (Pereiro et al., 2015). There are four identified NK-lysin genes in zebrafish: nklA, nklB, nklC and nklD (Pereiro et al., 2015). Only nklA and nklD are upregulated after SVCV challenge, indicating that these two genes may be involved in protecting against SVCV infection (Pereiro et al., 2015). Future studies are required to identify their anti-SVCV effects.

As studies on the regulatory mechanisms of fish antiviral immune signalling pathways remain far behind those of mammals, research in other laboratory animal models and at the translational level is needed to investigate the precise regulatory mechanisms of these proteins in immune pathways. Furthermore, high-throughput sequencing should be conducted to identify novel genes related to antiviral immunity.

**Diagnosis and prevention**

As SVCV has a significant impact on carp aquaculture, its rapid detection and identification are crucial for effective...
control of the disease. Conventional serological techniques used to detect SVCV include the virus neutralization test, immunoperoxidase assay, indirect immunofluorescence assay and ELISA. However, these techniques are laborious and time consuming (Ahne, 1981; Faisal & Ahne, 1984; Way, 1991; Rodák et al., 1993). Moreover, the indirect immunofluorescence assay and ELISA appear to cross-react with other rhabdoviruses (Way, 1991), leading to possible false-positive diagnoses. mAbs are also useful tools for detecting SVCV and studying the function of virus-specific proteins (Chen et al., 2008; Luo et al., 2014; Li et al., 2015). However, because the production of mAbs by traditional hybridoma technology is complicated and requires special skills, alternative methods which are simple and rapid for the production of mAbs are needed. Recently, a single-chain fragment variable antibody against SVCV has been developed using phage display technology and employed for rapid detection of SVCV (Liu et al., 2013). This antibody reacted specifically with SVCV, but did not cross-react with other viruses. Thus, this approach provides the basis for establishing simple and cost-effective way for the development of immunological detection assays for SVCV.

Various PCR-based assays have also been used to detect SVCV owing to their high sensitivity. These include reverse transcription (RT)-PCR combined with nested PCR (Koutna et al., 2003), multiplex real-time quantitative RT-PCR (Liu et al., 2008a) and one-step TaqMan real-time quantitative RT-PCR (Yue et al., 2008). These assays have clearly improved the specificity and sensitivity of detection (Kim, 2012). Although PCR is generally considered impractical for routine diagnostics in the field owing to the need for specialized instruments, skilled operators and isolation of RNA extracts, a recent report described an improved RT-PCR assay that was able to accurately detect SVCV directly from fish tissues, indicating the potential application of this technology for SVCV detection in infected fish in the field (Shimahara et al., 2015).

A loop-mediated isothermal amplification (LAMP) assay has been increasingly used for the detection of viruses, bacteria and parasites of humans, animals, and plants (Shen et al., 2014; Fallahi et al., 2015; Mohandas et al., 2015; Wang et al., 2015). RT-LAMP is a relatively simple and effective technique that can rapidly amplify specific nucleic acid sequences under isothermal conditions. Moreover, it requires uncomplicated and inexpensive equipment that can be easily manipulated at fish farms. This assay has been successfully applied for disease control in aquaculture (Khunthong et al., 2013; Zhang et al., 2014). Two studies have used RT-LAMP to detect SVCV based on nucleotide sequences of the G and M genes (Shivappa et al., 2008; Liu et al., 2008b). RT-LAMP can provide higher specificity and sensitivity than nested RT-PCR and shows excellent agreement with the standard virus isolation method (Liu et al., 2008b).

Recent advances in neuroscience have made a significant impact on a number of scientific domains, particularly biodiagnostics, where nanoparticle-oriented assays have been introduced for the detection of biomolecules. Single- or double-stranded oligonucleotides can be hybridized with gold nanoparticles in a colloidal solution under specific conditions (Lu et al., 2007). Saleh et al. (2012) exploited this phenomenon to develop a specific hybridization assay for direct detection of SVCV from clinical specimens without prior amplification of viral RNA (Saleh et al., 2012). The specificity of the assay was 100% when compared with positive and negative nested RT-PCR samples (Saleh et al., 2012). This assay offers several advantages over PCR-based assays, as it does not require a thermal cycling instrument and has a shorter test time (only 15 min) (Saleh et al., 2012).

The development of diverse and susceptible fish cell lines is also necessary for detection, isolation, and characterization of SVCV. SVCV has been reported to infect and multiply in various fish cell lines, including epithelioiama papulosum cyprinid cells from carp (Gotesman et al., 2015); skin cells from Ussuri catfish (Pseudobargus ussuriensis) and red-spotted grouper (Epinephelus akaara) (Lei et al., 2014; Ou et al., 2012); heart cells from giant grouper (Epinephelus lanceolatus) (Guo et al., 2015); haploid embryonic cells from medaka (Oryzias latipes) (Yuan et al., 2013); muscle and fin cells from bluefin trevally (Caranx melampygus) (Zhao & Lu, 2006); swim bladder, fin and snout cells from grass carp (Lu et al., 1990); and muscle, heart and swim bladder cells from snakehead (Channa striatus) (Zhao et al., 2004). SVCV propagation in these cells has been analysed by RT-PCR, electron microscopy, immunofluorescence assay and 50% TCID50 assays. The data obtained suggest that established cell lines can potentially serve as a useful tool for the isolation and detection of SVCV.

Vaccination strategies

The basic strategies for controlling SVCV involve strict hygienic measures and extermination of SVCV-infected fish (Ahne et al., 2002). Curative and preventive approaches to combat SVCV have been ineffectual and no commercialized SVCV vaccine is currently available. Inactivated virus vaccines against SVCV have provided limited protection (Tesarcik et al., 1978; Tesarcik & Macura, 1981), whereas attenuated vaccines have not been pursued because of a number of limitations, including improper attenuation of the virus, lack of quantitative assessment for the protection provided by the vaccine, and restrictive market and legal regulations (Fijan, 1984).

Antiviral DNA vaccines carrying a gene of a major antigenic viral protein have gained attention as an enticing alternative to traditional vaccines for a number of reasons, including thermostability, uncomplicated design and construction, prolonged shelf life, and the inability of the virus to recover its virulence (Jechlinger, 2006). The limitations of DNA vaccines that are currently being addressed include appropriate promoter selection, delivery
technologies and regulatory approvals (Pachuk et al., 2000). Regardless of these obstacles, research on DNA vaccines against fish viruses has increased steadily over the last decade (Chang et al., 2014; Ogas Castells et al., 2015; Chen et al., 2015a).

The earliest SVCV DNA vaccines exhibit a lower protection rate compared with other fish DNA vaccines against novirhabdoviruses, including IHNV and VHSV (Lorenzen & LaPatra, 2005). Therefore, multiple trials have been conducted to develop an equally efficacious SVCV DNA vaccine. The G gene of fish rhabdoviruses encodes a surface glycoprotein that is considered a major antigen for inducing a primary host immune response and is the most common gene used in DNA vaccine constructs (Kanellos et al., 2006; Emmenegger & Kurath, 2008). Kanellos et al. (2006) tested 10 SVCV DNA vaccine constructs carrying the SVCV G gene in carp. The majority of treated groups of carp showed little protection, with relative survival ranging from −11 to 33%. The strongest protection (48% relative survival) was observed in a group injected with a combination of two constructs expressing the full-length G gene; however, the relative contribution of the two constructs to this protection rate was not determined (Kanellos et al., 2006). The efficacy of another DNA vaccine that consists of a construct expressing the G gene of a North American SVCV isolate was investigated in ornamental koi and goldfish (Emmenegger & Kurath, 2008). In all trials, immunized fish demonstrated a strong protective response against SVCV, with relative survival ranging from 50 to 88% (Emmenegger & Kurath, 2008). Together, these studies provide validation for the potential use of G gene DNA vaccines as a prophylactic therapy in SVCV-susceptible fish stocks.

Although DNA vaccination via the parenteral route has been used as an efficient strategy to elicit antiviral immunity, it is not convenient for larger-scale immunization because of the handling stress on the fish as well as high labour and production costs (Adelmann et al., 2008). Furthermore, there is limited scientific literature about the fate of DNA vaccines after injection into fish (Tonheim et al., 2008). Mucosal tissues of fish, such as the intestine, gills and skin, are considered important for protection against invading pathogens (Costes et al., 2009; Gomez et al., 2013). Activation of mucosal immunity prevents pathogen infection and replication at the mucosa, whereas intramuscular vaccines fail to do this (Gomez et al., 2013). Oral vaccination is an effective way to induce mucosal immunity (Chen, 2000) and this strategy has shown a successful induction of the antiviral response against viral diseases in different fish species (Min et al., 2012). Ideally, oral vaccines must pass safely through the stomach and should be digested in the anterior segment of the intestine. Therefore, development of efficient vectors for delivery of vaccine antigens could offer a useful approach to vaccination against SVCV.

The potential for oral vaccines to contribute to fish health is tremendous. Steady progress in the development of commercial oral vaccines for aquaculture has addressed major hurdles specific to the oral delivery route (Dhar et al., 2014). The most important of these hurdles are the stability of immune-stimulating agents in the gut and their ability to successfully elicit a strong humoral immune response upon oral delivery (Dhar et al., 2014). Although the strong mucosal immunity elicited by oral vaccines is important, an effective vaccine should induce innate, mucosal and humoral immune responses. All types of vaccines, such as nucleic acid vaccines, subunit vaccines and inactivated or attenuated virus vaccines, should be effective as oral vaccines. To date, only a few studies have been conducted for the development of SVCV vaccines, and additional efforts are greatly needed to adequately prepare for large-scale outbreaks.

Lactobacilli possess multiple properties that make them suitable candidates for vaccine antigen delivery vectors (Galdeano & Perdigon, 2006). Lactobacilli are also known to induce a non-specific immunoadjuvant effect (Perdigon et al., 1991). Recently, a genetically engineered Lactobacillus plantarum co-expressing SVCV G protein and koi herpesvirus ORF81 protein was investigated for protective immunity in carp and koi through oral vaccination (Cui et al., 2015). Immunized carp and koi showed effective protection rates of 71 and 53%, respectively, at 65 days post-challenge (Cui et al., 2015). Moreover, immunized carp showed significantly elevated expression of immunoglobulin M (Cui et al., 2015). These results demonstrate the ability of recombinant Lactobacillus plantarum to induce a protective immune response in fish against SVCV and koi herpesvirus, and suggest a practical multivalent approach for large-scale control of spring viraemia of carp and koi herpesvirus disease (Cui et al., 2015).

Conclusion and future aspects

Intensive aquaculture has developed rapidly in many parts of the world, much faster than any other food animal production industry. To maintain high levels of aquaculture production, viral fish diseases need to be effectively controlled, as viral epidemics can often lead to high mortality and huge economic losses. Current strategies to prevent and control fish viral diseases have consisted of preventing the spread of viral pathogens from infected areas, destroying all fish at affected farms, and in some cases vaccination. Effective treatment of fish viral diseases has remained an unreachable goal in aquaculture. RNA interference, a new technique in the field of drug discovery and development, has shown some promise for protection against spring viraemia of carp in vitro (Gotesman et al., 2015), and warrants future study using in vivo models. A recently developed oral vaccine against SVCV can induce protective mucosal immunity and has overcome the limitations of injectable DNA vaccines (Cui et al., 2015). As mucosal tissues in fish are particularly important for protection against pathogen invasion (Gomez et al., 2013), additional studies on the fish mucosal immune system, as well as the design of rational mucosal vaccines and other immunotherapies, are important avenues for future investigation.
Much remains to be investigated in the areas of SVCV gene regulation, cell invasion and immune evasion strategies. It is clear from the information presented in this review that there are definite gaps that must be bridged between our current understanding of immune responses to SVCV and the specific mechanisms by which SVCV causes pathogenesis. Comprehensive analysis of SVCV in the context of its host immune system can provide a deeper insight into viral invasion strategies as well as host immune countermeasures.

The role of specific amino acid substitutions is well known in viral pathogenesis. Many viruses, including Usutu virus, West Nile virus, Japanese encephalitis virus, influenza virus and enterovirus, exhibit altered pathogenesis that is due to amino acid substitutions at specific positions in viral proteins (Chambers et al., 1998; Ashraf et al., 2015; Gromowski et al., 2015; Han et al., 2015; Kataoka et al., 2015). This phenomenon has also been documented in the fish virus VHSV, showing that a single amino acid substitution in the L protein resulted in the change of viral virulence to rainbow trout gill epithelial cells (Kim et al., 2014). However, the importance of these amino acid substitutions in SVCV pathogenesis remains unclear. Our comparative amino acid sequence analysis of completely sequenced SVCV strains identifies distinct amino acid substitutions that may provide characteristic features to particular strains. To gain a better knowledge about the potential roles of SVCV strain-specific amino acid substitutions, comparative pathogenesis studies on different SVCV strains using fish models will be important.

Acknowledgements

This work was jointly supported by Natural Science Foundation of China (31172433) and Fundamental Research Funds for the Central Universities (2013PY071).

References


Spring viraemia of carp virus


