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Contents

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REVIEW

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REVIEW

Reverse genetics: Unlocking the secrets of negative sense RNA viral pathogens

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Abstract

Negative-sense RNA viruses comprise several zoonotic pathogens that mutate rapidly and frequently emerge in people including Influenza, Ebola, Rabies, Hendra and Nipah viruses. Acute respiratory distress syndrome, encephalitis and vasculitis are common disease outcomes in people as a result of pathogenic viral infection, and are also associated with high case fatality rates. Viral spread from exposure sites to systemic tissues and organs is mediated by virulence factors, including viral attachment glycoproteins and accessory proteins, and their contribution to infection and disease have been delineated by reverse genetics; a molecular approach that enables researchers to experimentally produce recombinant and reassortant viruses from cloned cDNA. Through reverse genetics we have developed a deeper understanding of virulence factors key to disease causation thereby enabling development of targeted antiviral therapies and well-defined live attenuated vaccines. Despite the value of reverse genetics for virulence factor discovery, classical reverse genetic approaches may not provide sufficient resolution for characterization of heterogeneous viral populations, because current techniques recover clonal virus, representing a consensus sequence. In this review the contribution of reverse genetics to virulence factor characterization is outlined, while the limitation of the technique is discussed with reference to new technologies that may be utilized to improve reverse genetic approaches.

Key words: Reverse genetics; Viral pathogen; Negative sense RNA viruses; Influenza A virus; Ebola virus; Rabies virus; Hendra virus; Nipah virus

Core tip: Several negative sense RNA viruses are capricious, pandemic threats and give no quarter to their human hosts. Reverse genetic approaches have been valuable for discovery of key virulence factors mediating disease with the aim of treatment and vaccine development, and knowledge acquisition to genetically map pathogenic potential. Despite the value of the reverse genetics approach current systems are limited by molecular cloning procedures that do not enable reproduction of genetically heterogeneous virus populations that circulate in nature. Advances in molecular biology may facilitate production of genetically diverse viral populations that better represent natural isolates.

Edenborough K, Marsh GA. Reverse genetics: Unlocking the secrets of negative sense RNA viral pathogens. *World J Clin Infect Dis* 2014; 4(4): 16-26 Available from: URL: http://www.wjgnet.com/2220-3176/full/v4/i4/16.htm DOI: http://dx.doi.org/10.5495/wjcid.v4.i4.16

INTRODUCTION

Rational design of vaccines and antiviral therapies is facilitated by discovery of viral pathogenicity factors, the viral genes and proteins producing disease. Negative-sense RNA viruses are comprised of formidable human and zoonotic pathogens consisting of seven viral families; four are characterized by non-segmented genomes (Filoviridae, Rhabdoviridae, Paramyxoviridae and Borna-

viridae), while the remaining three are distinguished by segmented genomes (Orthomyxoviridae, Bunyaviridae and Arenaviridae)[1]. Before 1994, when pioneering experiments enabled recovery of the first negative-sense RNA virus from cloned cDNA[2], *in vivo* serial virus passage, often at suboptimal temperatures, was the main method utilized to generate pathogenic variants[3] and retrospective sequence analysis of viral genes enabled associations between genes and pathogenesis[4]. Today reverse genetics is routinely employed to manipulate viral genomes for the purpose of viral pathogenesis research. Briefly cDNAs, representing the full-length RNA genome/genome segments, are cloned into vectors containing T7 RNA polymerase (T7) or RNA polymerase I (pol I) transcriptional units. Transfection of these plasmids, in concert with viral polymerase complex expression, into permissive cells facilitates transcription of viral mRNAs, full length vRNA and recovery of infectious virus[5].

OPTIMIZING CONDITIONS FOR REVERSE GENETICS

Minigenome assays

Reverse genetic systems have been optimized for individual viruses by use of minigenomes[6]; open reading frames (ORFs) of reporter constructs encoding bioluminescent enzymes or fluorescent proteins are inserted in between viral noncoding sequences that are sufficient for the transcription and replication activity of the viral polymerase[7] (Figure 1A). Leader and trailer sequences at the respective 3' and 5' ends of the vRNA are critical for viral polymerase activity, and hence reporter expression, as demonstrated in the case of Marburg virus[8] and Ebola virus[9,10]. Likewise minigenome constructs for Influenza virus, Orthomyxoviridae, include the 5' and 3' noncoding regions of one of the eight vRNAs such as nucleoprotein (NP)[11] or non-structural[12] segments. In the case of Paramyxoviruses, however, the addition of gene start and gene end sequences in combination with leader and trailer sequences have been shown to enhance mRNA production[13], while it may also be important for the number of nucleotides of the minigenome to be perfectly divisible by six as each nucleocapsid (N) protein is thought to interact with six nucleotides[14].

For expression of reporter genes from RNA transcripts, produced from minigenome constructs, viral polymerase complexes are supplied *in trans*. Eukaryotic expression vectors such as pCAGGS contain strong promoters such as CAG, chicken β actin fused to a cytomegalovirus enhancer, and transient transfection of polymerase constructs promote sufficient viral protein expression to elucidate the minimum number of viral proteins required for reporter expression. For Influenza virus minigenome activity proteins, which form ribonucleoprotein complexes (RNPs), are required including polymerase basic 2 (PB2), PB1, acidic polymerase (PA) and NP proteins[15]. Likewise for Rhabdoviridae[16] and Paramyxoviridae[14] members plasmids encoding N, phosphoprotein (P) and large polymerase (L) are co-transfected with the minigenome to enable reporter expression. The minimal number of proteins required for minigenome activity may vary considerably even within one virus family, *e.g.*, RNA transcription of Respiratory syncytial virus, Paramyxoviridae, was augmented with the inclusion of matrix (M) 2 protein[17], while addition of M protein to Measles virus (MV) minigenome assays reduced reporter expression by reducing vRNA synthesis[18]. Similarly, the expression of accessory proteins may inhibit minigenome activity and repression of some of these proteins may be required for measurement of any polymerase activity[19]. The need for different protein combinations for reporter activity underscores the importance of minigenome assays in determining functional associations between viral proteins for viral mRNA transcription.

PolI and II systems: Transcription in the nucleus

Selection of promoters that drive RNA transcription from minigenome constructs is dependent upon whether viral transcription occurs in the nucleus or the cytoplasm during natural replication of the virus. T7-dependent systems may be more suitable for viruses that replicate RNA within the cytoplasm, while polII systems may better mimic viral replication cycles that involve transcription in the nucleus, however recent studies have indicated some exceptions to this view. Infectious Uukuniemi[20], Influenza[21], Thogoto[22], Borna disease virus, MV[23] and Ebola virus[10] have all been successfully recovered from cloned cDNAs by the use of cellular RNA polymerases such as pol I (Figure 1B). The conventional role of pol I is to transcribe ribosomal RNAs without addition of 5'caps and 3' poly-A tails[24], therefore it is a suitable host enzyme for the processing of viral RNA molecules generating well defined vRNA 3' and 5' termini[25]. To employ RNA pol I a cDNA copy corresponding to each viral segment, or a full length cDNA molecule, is placed between a pol I truncated promoter and a pol I terminator enabling synthesis of vRNA[26]. Interaction between RNA pol I and its promoter is species-specific, therefore promoter sequence is carefully selected to suit the cell line destined for virus rescue[27,28].

RNA pol Ⅱ cytomegalovirus promoters have also been utilized to initiate transcription of viral messenger RNA for Influenza virus rescue systems[26] and also has been shown to enhance cRNA expression for MV recovery in relation to other reverse genetic systems, dependent upon T7, however as pol Ⅱ transcripts may be spliced and polyadenylated the utility of pol Ⅱ for virus rescue of other negative-sense RNA viruses is still to be determined[23].

T7 systems: Transcription in the cytoplasm

T7 polymerase has been particularly useful for recovery of negative-sense RNA viruses, which mostly undergo transcription in the cytoplasm, including Hendra[29], Nipah[30], MV[31], Sendai[32], Rabies[2], Ebola[33], Marburg[34], Newcastle disease virus[35], RSV[17], Vesicular stomatitis vi-

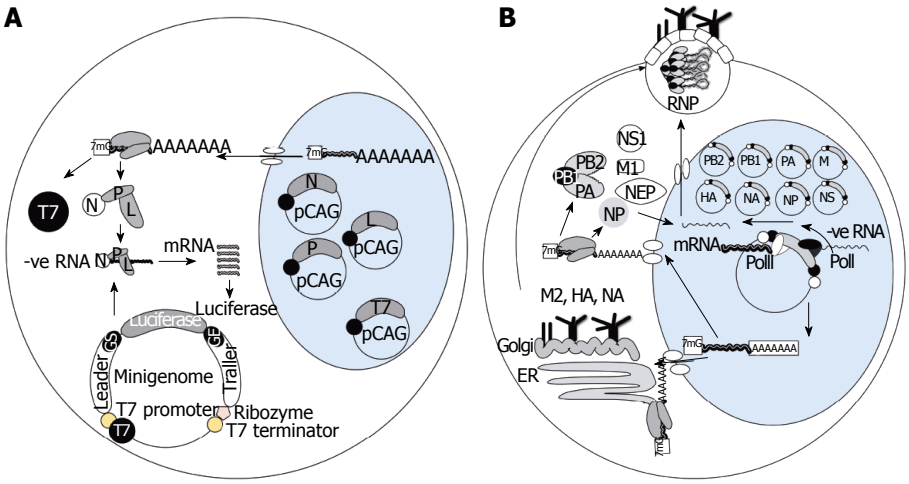

Figure 1 Reverse genetic approaches for the study of viral pathogenesis. This figure demonstrates a T7 driven minigenome system (A) and a polI dependent virus rescue system for influenza A virus (B). In (A) the pCAGGS plasmids enable synthesis of T7 polymerase and the viral polymerase complex; nucleoprotein (N), phosphoprotein (P) and large polymerase (L). T7 drives transcription of the minigenome for intracellular synthesis of negative-sense RNA (-veRNA) genome analogues. The minigenome contains Luciferase flanked by leader and trailer sequences that contain gene start (GS) and gene end (GE) sequences essential for polymerase function. To ensure correct processing of the trailer sequence the hepatitis δ ribozyme is included prior to the T7 terminator sequence. The viral proteins encapsidate the RNA analogue and facilitate transcription, which results in Luciferase production and increase in luciferase indicates minigenome transcription. In (B) eight plasmids PB1, PB2, PA, HA, NP, NA, M, NS, representing the influenza A virus genome, are transfected into 293T cells and each of the negative sense viral RNA segments (-veRNA) are produced by poll driven transcription. From the same plasmid, viral messenger RNAs are transcribed in an ambisense direction by polII. Capped and polyadenylated mRNAs are exported from the nucleus into the cytoplasm. Some viral mRNAs are spliced before export, such as M2 and NEP, and translation of viral mRNA occurs on ribosomes attached to endoplasmic reticulum or on ribosomes free in the cytoplasm. Viral proteins in the cytoplasm return into the nucleus and facilitate replication of the viral genome, which is transcribed to produce more viral mRNA and exported from the nucleus. Viral membrane proteins are modified and transported to the apical membrane of the cell. The RNPs are transported to the plasma membrane containing HA and NA in association with M1 and NEP, and 8 RNPs are packaged into progeny virions for neuraminidase-mediated release. HA: Hemagglutinin; NA: Neuraminidase; PB: Polymerase basic; PA : Acidic polymerase; RNPs: Ribonucleoprotein complexes; NS: Non-structural.

rus[36] and Lymphocytic choriomeningitis virus[37]. Investigators have had more success in virus recovery by insertion of full-length antigenome, rather than genome sense, between T7 promoter and terminator sequences[2]. The T7 promoter can be modified to enhance transcription initiation and reporter expression in minigenome assays by the addition of more than two G nucleotides[38], while if added in combination with a nuclear localization signal the T7 system has enabled recovery of Influenza virus[39]. The disadvantage of T7 systems, in contrast to polI, includes necessary sequences bordering the 5' and 3' antigenome ends to form autocatalytic ribozymes that cleave nonviral terminal nucleotides added during transcription. Early rescue systems focussed on correct processing of the RNA 5' ends, or trailer sequence[38,40], by insertion of an adjacent Hepatitis delta virus (HDV) sequence before the T7 terminator sequence, and the HDV ribozyme sequence has recently been optimized for more efficient vRNA cleavage[41]. Enhanced recovery of infectious virus has also been documented by addition of a hammerhead ribozyme sequence prior to the 3' leader[41].

An advantage of T7 dependent systems includes transfectable cell lines of several species can be employed for the purpose of virus recovery, providing supplementation with an exogenous source of T7[39]. Choice of cell may be of value when vaccine approved cell lines must be used, or in the case of zoonotic viruses that have limited cell tropism. In early reverse genetics systems cytoplasmic T7 was supplied by addition of recombinant vaccinia virus[42], however its cytopathic effects have been found to impede virus recovery and necessitates plaque purification for removal of vaccinia from the virus culture[43]. These issues have been overcome by use of modified vaccinia Ankara strains[44], however more practical systems are now accessible such as T7 expression plasmids that can be transiently transfected[45] or stably transfected[46,47] into permissive cell lines.

REVERSE GENETICS AND PATHOGENICITY FACTOR DISCOVERY

Influenza A virus

Influenza A virus, a member of *Orthomyxoviridae* family, contains eight negative-sense RNA segments each corresponding to one of eight viral genes; two of these, the hemagglutinin (HA) and neuraminidase (NA) encode the surface glycoproteins that protrude from the viral envelope. Currently 17 HA and 10 NA types have been identified and all but the most recently described subtype (H17N10 from bats) have been isolated from aquatic birds such as waterfowl and shorebirds, which act as a natural reservoir for the virus[48,49]. The only subtypes circulating in humans, H3N2 and H1N1, cause mild disease associated with viral replication in the upper respiratory tract and large airways[50,51], while replication in the lower respiratory tract[52] or sites outside the respiratory tract results from infection with virulent isolates, such as

Table 1 Outline of viral proteins that contribute to virulence as determined by reverse genetics

Virus	Virulence factors	Role in pathogenesis
Influenza A	Multibasic cleavage site	Facilitates viral spread to cells outside the respiratory tract
Ebola	Glycoprotein	Transmembrane form mediates host cell attachment and its soluble forms activate mononuclear phagocytes and endothelial cells
	Viral protein 24 and nucleoprotein	Antagonists of IFN responses
	Viral protein 35	Viral polymerase cofactor that suppresses RIG-I like receptor signalling
Rabies	Glycoprotein	Neurotropic surface glycoprotein that facilitates spread to the brain
	Phosphoprotein	Viral polymerase cofactor and antagonist of IFN responses
Hendra and Nipah	Phosphoprotein	Viral polymerase cofactor and antagonist of IFN responses
	V and W proteins	Antagonists of IFN responses
	C protein	Regulates viral transcription and affects activation of innate immune cells

IFN: Interferon.

highly pathogenic avian isolates that infect humans *via* inter-species transmission events[53].

The virulence factors enabling viral spread beyond the respiratory tract have been characterized in reverse genetic studies. Influenza virus reverse genetic systems have been thoroughly optimized since their initial iterations when purified RNP and RNA were transfected *in vitro* and recovered with the addition of helper viruses[54]. In early plasmid-based reverse genetic systems influenza viral RNA synthesis was dependent upon supplementation of additional expression plasmids for NP and the polymerase complex, PB1, PB2 and PA, *in trans*[21,55], however shrewd optimization by inclusion of an RNA pol Ⅱ transcriptional unit on the same plasmid as the RNA pol Ⅰ promoter, in an ambisense direction, generated viral mRNA molecules *in cis* enabling production of viral protein and vRNA from a single plasmid[56]. More recently gene segments were concatenated onto a single cassette that encoded multiple segments each separated by a transcriptional unit with the aim to hasten the recovery of vaccine seed viruses[57], which is essential for production of pandemic vaccines.

One particular virulence factor that has been well characterized in reverse genetic studies includes the HA glycoprotein, which interacts with terminal sialic acids for host cell attachment[58] and orchestrates fusion of the viral envelope and endosomal membrane of the cell for release of RNPs into the cytoplasm[59]. For efficient fusion the HA precursor must be cleaved at a prominent loop to form two subunits, HA1 and HA2, cleavage of the HA glycoprotein is a process essential for multiple rounds of viral replication and is carried out by enzymes that are produced by the host[60,61]. For most human seasonal influenza and Low Pathogenicity Avian Influenza viruses the cleavage occurs at the site of a single arginine (R) residue[60,62]. For this reason, these viruses are limited to tissues that contain host enzymes with the corresponding recognition preference for single basic amino acids[60,63].

In the HA of highly virulent subtypes insertions of multiple basic amino acid residues have been found and this region has been coined the multi-basic cleavage site (MBCS, Table 1)[64,65]. The role of the MBCS in pathogenesis for chickens was assessed by generating H5N2 mutant viruses containing variable sequence lengths and combinations of basic amino acid residues in the HA *via* reverse genetics[66]. In this study they found association between presence of a > 4 basic amino acid residues in the cleavage site, efficient HA cleavage in chicken embryonic fibroblasts and lethality to chickens, which was caused by spread of virus to brain *via* neurons and systemic organs *via* the blood stream. More recent plasmid based reverse genetic studies have demonstrated a similar role for the MBCS in viral pathogenesis for mammals, such as mice[67] and ferrets[68,69], which corresponds to the pathological and clinical observations of humans infected with highly pathogenic influenza such as detection of virus in systemic organs of fatal H5N1 cases[70] and detection of vRNA in the blood stream of infected patients[71]. Since the importance of the MBCS for influenza pathogenesis has been established recent research has focused on reducing replication of Influenza virus with substrate-analogue peptide mimetic inhibitors that target host cell proteases, specifically those carrying out HA0 cleavage[72]. This is a successful example of the use of reverse genetics for identification of a virulence factor, the MBCS, and production of an inhibitor based on the understanding of virulence mechanisms.

Ebola virus

In 1976 Ebola virus, a single-stranded negative-sense RNA virus of 18.9kb within the Filoviridae, first emerged in humans and thereafter several outbreaks in Sudan, western and central Africa have been documented[73]. Index cases are often associated with butchering, handling or consuming bush meat such as fruit bats[74], the potential natural reservoir of the virus[75], and also close contact with non-human primates[76]. Human-to-human transmission occurs *via* close contact[77] and long incubation periods, prior to symptom development, facilitate viral spread in the community causing stigmatization of health care workers and relatives of the sick[78]. Ebola viruses isolated from different geographical locations in Africa have caused similar disease symptoms and signs such as headache, myalgia, muscle spasms, fever, malaise, abdominal pain, haemorrhage and maculopapular rash[79], although the latter was more commonly noted in infected patients

of the Zaire outbreak, which of all the outbreaks, has the highest documented case fatality rate[80,81]. In contrast humans infected with the Reston Ebola virus isolate, which emerged *via* importation of infected monkeys from the Philippines into Reston, Virginia, United States, developed antibodies to the virus in the absence of clinical disease[82] indicating this virus isolate was not pathogenic, although only a small number of humans were exposed. Reston Ebola virus has since been detected in piggeries in the Philippines while serological studies suggest a small number of pig farm workers have been infected with the virus[83].

Both T7 and pol I dependent systems have been utilized for minigenome assays to characterize the viral proteins mediating transcription for Zaire and Reston Ebola viruses, and to recover infectious viruses for several Ebola virus isolates[84]. Reverse engineered viruses have been used to assess the role of the Ebola virus glycoprotein (GP, Table 1), which forms trimeric spikes on the viral envelope and mediates host cell attachment and entry[85]. Produced through a process of transcriptional RNA editing[86], GP protein expression is regulated in infected cells[87]; secretory GP is produced from unedited transcripts, while transmembrane GP is produced from edited transcripts that preside at lower frequency[88]. GP expression is cytotoxic acting to increase the permeability of venous and arterial blood vessels, compromising vascular function[89]. Recently chimeric Ebola viruses, in which the GP of Zaire and Reston virus isolates were exchanged, have been utilized to clarify isolate-specific differences in virulence[90]. Interferon-α/β receptor knock-out (IFNAR$^{-/-}$) transgenic mice were selected to characterize *in vivo* pathogenicity of the chimeric viruses, as the need for virus adaptation *via* serial passage is unnecessary, unlike immune competent mice. Reverse engineered Reston virus was not pathogenic to IFNAR$^{-/-}$ mice and only replicated to low levels in liver and spleen, which mirrors the absence of human disease[90]. In contrast, Zaire virus caused rapid weight loss and replicated to high titres in spleen and liver, which indicated that the IFNAR$^{-/-}$ mouse model recapitulated human disease observations in relation to both virus isolates[90]. Interestingly, introduction of the Reston GP into the Zaire virus did not attenuate virulence in IFNAR$^{-/-}$ mice, suggesting that Zaire GP is not the only determinant of virulence, and instead the robust replicative capacity of Zaire virus *in vitro* and *in vivo* was consisted with its virulence for IFNAR$^{-/-}$ mice[90].

Other factors for Zaire Ebola virus virulence have been identified *via* serial passage of reverse engineered viruses in mice and include viral protein 24 and nucleoprotein (Table 1), factors that are involved in evasion of host type 1 interferon responses[91]. Other components of the innate immune response, particularly transcription factors such as interferon regulatory factor 3, may be suppressed by viral protein 35 (VP35, Table 1), a cofactor of Ebola viral polymerase[92] and production of reverse genetic viruses containing mutations in VP35 have been used in transcriptome studies[93], which may provide key insights into virus host interactions.

Rabies virus

Rabies virus, a member of the Rhabdoviridae, was the first single-stranded negative-sense virus recovered by reverse genetics[2]. Wildlife host reservoirs of rabies virus include bats[94], racoons and foxes[95] although cross-species transmission to non-human primates and domestic animals such as dogs and cats perpetuate human disease[96-98]. Risks for human infection include direct exposure to saliva shed from rabid animals occurring *via* animal bite or contamination of broken skin[99]. Human disease results from fatal encephalitis that progresses as virus spreads to the central nervous system *via* retrograde transport along axons of peripheral nerves, which is mediated by rabies glycoprotein (G, Table 1)[100]. The 12kb long genome encodes five proteins; nucleoprotein (N), phosphoprotein (P, Table 1), matrix protein (M), G and the RNA-dependent RNA polymerase (L). Viruses produced by reverse genetics have elucidated that G, M and P proteins play important roles in the severity of rabies disease by either facilitating cell to cell spread[101] or antagonizing host innate immune responses[102].

Currently rabies virus vaccines delivered to humans are beta-Propiolactone inactivated and administered intramuscularly in three doses to generate neutralizing G-specific antibody titres, which wane overtime, as such additional booster shots may be needed[103]. Due to the requirement for multiple doses and the high cost of vaccination and post-exposure prophylaxis (PEP) therapies more than 55000[104] rabies-related deaths are still reported annually predominantly in developing countries. Replication deficient rabies viruses (RDRV) produced *via* reverse genetics may be a low cost alternative to current vaccines and PEP. RDRV have been produced by T7 driven reverse genetic systems such that the viruses contained neither coding capacity for M nor P, instead the latter encoded two copies of G for protein overexpression and induction of greater G specific immune responses[105]. RDRV vectors have also been shown to be immunogenic demonstrated by the induction of neutralizing G-specific antibodies in non-human primates following a prime boost immunization strategy[106]. Safety is a main concern for the use of live attenuated viral vaccines, but because RDRV vectors replicate in cell culture only in the presence of P or M protein supplementation and are innocuous to immune deficient transgenic mice[106] the risk of reversion may not be as great as other live attenuated vaccine viruses that are attenuated through single amino acid changes. Rabies virus has also been shown to be a safe and excellent vaccine vector with the ability to generate antibody responses targeting human immunodeficiency virus envelope proteins, severe acute respiratory syndrome coronavirus and hepatitis C virus proteins[107]. Collectively, these RDRV studies have highlighted the diverse utility of reverse genetics, not only enabling discovery of virulence determinants, but

also applying our understanding of virulence to rationally engineer attenuated viruses for the purposes of vaccination.

Hendra and Nipah viruses

Hendra virus (HeV) and Nipah virus (NiV), classified within the Henipavirus genus of the Paramyxoviridae, which are harboured asymptomatically by Pteropid fruit bats[108], were identified as aetiological agents of severe human infections that occurred in the 1990's. Inter-species transmission occurs *via* intermediate hosts; infected horses were associated with HeV human cases in Australia[109] and infected pigs were associated with NiV cases in Malaysia[110]. Additional risk factors for contraction of Nipah virus in Bangladesh have included consumption of raw date palm sap, a delicacy in Bangali culture, contaminated by bat urine or saliva[111], while limited human-to-human transmission has been documented in cases of very intimate contact such as preparing an infected corpse for burial[112]. In humans HeV and NiV infections are associated with high case fatality rates and severe disease. NiV infected humans develop respiratory and neurological signs such as dyspnoea, disorientation, confusion and muscle spasms that are associated with the expansive tissue tropism of the virus, which includes infection of neurones of the central nervous system, endothelium, lymphoid and respiratory tissues[113]. In 10% of infected people NiV has been shown to reside in a quiescent form for months or years until the virus reactivates causing fatal neurological disease[114]. HeV disease in humans has not been thoroughly characterized as few human infections have occurred, however from limited reports of post-mortems and disease signs it appears the respiratory and neurological disease caused by HeV is akin to that caused by NiV infection[115,116].

NiV and HeV have non-segmented genomes of ~18kb in length that encodes for more than six proteins and NiV and HeV are closely related to each other with amino acid sequence similarities of > 80% for many of the viral proteins[117]. Nucleocapsid protein (N), phosphoprotein (P), matrix protein (M), fusion protein (F), glycoprotein (G) and large polymerase (L) are encoded on discrete transcriptional units[118], while three accessory viral proteins are produced from the P gene (Table 1) including the C protein that is transcribed from an alternate open reading frame (ORF), and also V and W proteins that are produced by the addition of G nucleotides into transcribed mRNAs *via* RNA editing[119]. P, V, W and C proteins play important roles in infection by impeding activation of host antiviral responses. A conserved feature of the paramyxovirus V protein is its ability to bind melanoma differentiation-association gene 5 (MDA5), a pattern recognition receptor, impeding the recognition of dsRNA resulting in inhibition of IFN-β induction[120,121]. Furthermore, V and W proteins can prevent activation of the type I IFN signalling pathway by sequestration of signal transducer and activator of transcription (STAT) in the cytoplasm or nucleus, reducing STAT mediated induction of interferon stimulating genes key to innate antiviral responses[122]. Inhibition of various components of the antiviral response by several NiV and HeV proteins underscores the role of P, V, W and C for viral pathogenesis.

HeV and NiV reverse genetics systems have been successful in virus recovery with use of T7[29,30] dependent systems and co-transfection of the protein expression plasmids N, P and L, which encapsidate the RNA forming the RNP complex, as these proteins are essential for minigenome function[14]. The transfection ratios of N:P:L require optimization for efficient virus recovery as poor reporter expression in minigenome assays have been noted in the context of high concentrations of P protein[45], which likely results from the C protein inhibiting minigenome expression[19]. To further examine importance of C, V and W proteins for NiV pathogenesis several recombinant viruses were produced; Stop codons were introduced downstream of the C ORF site or the RNA editing site to prevent expression of C or V and W respectively. Despite these changes all viruses expressed functional forms of the P protein[123]. Following confirmation of P but not V, W or C expression in infected cells, it was determined that the recombinant viruses replicated efficiently *in vitro*, which indicated these proteins were not essential for viral replication. Virus pathogenicity was assessed by use of a hamster infection model, wherein it was demonstrated that suppression of C and V, but not W protein, completely attenuated NiV as weight loss, disease signs and high levels of viral replication in systemic organs were not observed[123]. Another study by a different group compared host responses in human endothelial cell lines infected with wildtype NiV verses one of the attenuated viruses, NiV lacking C protein expression (NiVΔC). With microarray analysis they established that compared to wildtype NiV, NiVΔC induced higher levels of cytokines and chemokines such as interleukin 1 beta (IL-1β), IL-8, CXCL2, CXCL3, CXCL6 and CCL20[124]. These findings indicated that NiV C plays a role in inhibiting induction of proinflammatory cytokines and recruitment of leucocytes and lymphocytes into sites of infection such as the lung. This study also examined the pathogenesis of NiVΔC in hamsters and the researchers were only able to partially replicate the attenuated phenotype of NiVΔC, as 30%-90% of animals required euthanasia following infection. The reason for the variation in results between the two studies is yet to be ascertained, however it highlights the limitations associated with insertion of silent restriction sites for the purposes of engineering mutations into plasmids, as laboratories will insert a different variety of silent mutations that may have unknown effects on the virulence of the virus.

REVERSE GENETICS TECHNOLOGIES HAVE LIMITED UTILITY FOR THE STUDY OF DIVERSE RNA VIRUS POPULATIONS

RNA viruses, HIV and Influenza virus in particular, spread between and within hosts as genetically hetero-

geneous virus populations, or quasispecies, clustering around a dominant virus sequence[125]. Quasispecies are perpetuated by spontaneous mutations afforded by low fidelity viral RNA polymerases, and although exact mutation rates may differ for each virus[126], they are in the range of 10^{-4} mutations per nucleotide copied[127], therefore 1.3 mutations would be expected to occur with every replication of the influenza genome of ~13kb. Quasispecies are thought to act cooperatively with the aim of facilitating viral persistence within hosts[125,128]. Genetic heterogeneity has been found important for poliovirus pathogenesis as demonstrated by the ability of a genetically diverse, but not a homogenous, virus population for invasion into the CNS[129]. HIV genetic diversity may also influence viral tropism and larger sequence diversity has been associated with faster disease progression[128]. With this in mind we are faced with a technological drawback; the requirement of producing infectious clones for the purpose of virus rescue also removes population heterogeneity that may play pivotal roles in pathogenesis. Overall, care should be taken in the selection of a consensus sequence to produce infectious clones representative of dominant and also subdominant variants.

CONCLUSION

Reverse genetic technologies have proven critical to study the contribution of viral genetic factors to disease severity by enabling production of well-defined, recombinant negative-sense RNA viruses, such as a mutant and wild-type viruses, which can be compared for the purpose of identifying chief virulence determinants in the context of host-pathogen systems. For several negative-sense RNA viruses effective rescue methods have been developed, which may be dependent upon either T7 or pol Ⅰ and Ⅱ transcriptional units. Furthermore, inclusion of polymerase proteins or 5' and 3' cleavage sequences for correct vRNA processing may also be necessary for rescue, although these conditions are optimized for each virus and minigenome assays have proved useful for this purpose.

Recombinant viruses, however, are produced by selection of a consensus sequence that forms the basis of the infectious clone and therefore recombinant viruses are likely to constitute only the dominant viral species of a potentially diverse natural virus population. Reduction and alteration in viral heterogeneity, as a consequence of reverse genetics, is a limitation not often taken into account in the context of pathogenesis studies. However, with the advent of next generation sequence technologies for thorough characterization of virus populations we stand in good stead to gather a better grasp of viral heterogeneity in a field isolate and molecular biologists may be capable of recapitulating diverse viral populations *via* reverse genetics. Recent technologies such as Gibson cloning[130] and barcoding virus populations[131] are likely to enable researchers to produce heterogeneous virus populations that can be studied for characterization of the pathogenic potential of diverse viral populations, with a particular focus on the importance of subdominant viruses for severe disease outcomes. Despite this limitation, reverse genetics enables production of viruses that may be utilized for various future applications such as live-attenuated vaccines, mapping neural pathways in the brain, oncolytic virus production and delivery of microRNAs as a therapy for viral infections.

REFERENCES

1 **Neumann G**, Whitt MA, Kawaoka Y. A decade after the generation of a negative-sense RNA virus from cloned cDNA - what have we learned? *J Gen Virol* 2002; **83**: 2635-2662 [PMID: 12388800]

2 **Schnell MJ**, Mebatsion T, Conzelmann KK. Infectious rabies viruses from cloned cDNA. *EMBO J* 1994; **13**: 4195-4203 [PMID: 7925265]

3 **Pearce JM**. Louis Pasteur and rabies: a brief note. *J Neurol Neurosurg Psychiatry* 2002; **73**: 82 [PMID: 12082056 DOI: 10.1136/jnnp.73.1.82]

4 **Hiromoto Y**, Saito T, Lindstrom S, Nerome K. Characterization of low virulent strains of highly pathogenic A/Hong Kong/156/97 (H5N1) virus in mice after passage in embryonated hens' eggs. *Virology* 2000; **272**: 429-437 [PMID: 10873787 DOI: 10.1006/viro.2000.0371]

5 **Bridgen A**, Elliott RM. Rescue of a segmented negative-strand RNA virus entirely from cloned complementary DNAs. *Proc Natl Acad Sci USA* 1996; **93**: 15400-15404 [PMID: 8986823 DOI: 10.1073/pnas.93.26.15400]

6 **Conzelmann KK**. Reverse genetics of mononegavirales. *Curr Top Microbiol Immunol* 2004; **283**: 1-41 [PMID: 15298166 DOI: 10.1007/978-3-662-06099-5_1]

7 **Hoenen T**, Groseth A, de Kok-Mercado F, Kuhn JH, Wahl-Jensen V. Minigenomes, transcription and replication competent virus-like particles and beyond: reverse genetics systems for filoviruses and other negative stranded hemorrhagic fever viruses. *Antiviral Res* 2011; **91**: 195-208 [PMID: 21699921 DOI: 10.1016/j.antiviral.2011.06.003]

8 **Mühlberger E**, Lötfering B, Klenk HD, Becker S. Three of the four nucleocapsid proteins of Marburg virus, NP, VP35, and L, are sufficient to mediate replication and transcription of Marburg virus-specific monocistronic minigenomes. *J Virol* 1998; **72**: 8756-8764 [PMID: 9765419]

9 **Mühlberger E**, Weik M, Volchkov VE, Klenk HD, Becker S. Comparison of the transcription and replication strategies of marburg virus and Ebola virus by using artificial replication systems. *J Virol* 1999; **73**: 2333-2342 [PMID: 9971816]

10 **Groseth A**, Feldmann H, Theriault S, Mehmetoglu G, Flick R. RNA polymerase I-driven minigenome system for Ebola viruses. *J Virol* 2005; **79**: 4425-4433 [PMID: 15767442 DOI: 10.1128/jvi.79.7.4425-4433.2005]

11 **Ozawa M**, Shimojima M, Goto H, Watanabe S, Hatta Y, Kiso M, Furuta Y, Horimoto T, Peters NR, Hoffmann FM, Kawaoka Y. A cell-based screening system for influenza A viral RNA transcription/replication inhibitors. *Sci Rep* 2013; **3**: 1106 [PMID: 23346363 DOI: 10.1038/srep01106]

12 **Moncorgé O**, Long JS, Cauldwell AV, Zhou H, Lycett SJ, Barclay WS. Investigation of influenza virus polymerase activity in pig cells. *J Virol* 2013; **87**: 384-394 [PMID: 23077313 DOI: 10.1128/jvi.01633-12]

13 **Kuo L**, Grosfeld H, Cristina J, Hill MG, Collins PL. Effects of mutations in the gene-start and gene-end sequence motifs on transcription of monocistronic and dicistronic minigenomes of respiratory syncytial virus. *J Virol* 1996; **70**: 6892-6901 [PMID: 8794332]

14 **Halpin K**, Bankamp B, Harcourt BH, Bellini WJ, Rota PA.

Nipah virus conforms to the rule of six in a minigenome replication assay. *J Gen Virol* 2004; **85**: 701-707 [PMID: 14993656 DOI: 10.1099/vir.0.19685-0]
15 **Huang TS**, Palese P, Krystal M. Determination of influenza virus proteins required for genome replication. *J Virol* 1990; **64**: 5669-5673 [PMID: 2214032]
16 **Conzelmann KK**, Schnell M. Rescue of synthetic genomic RNA analogs of rabies virus by plasmid-encoded proteins. *J Virol* 1994; **68**: 713-719 [PMID: 8289375]
17 **Collins PL**, Hill MG, Camargo E, Grosfeld H, Chanock RM, Murphy BR. Production of infectious human respiratory syncytial virus from cloned cDNA confirms an essential role for the transcription elongation factor from the 5′ proximal open reading frame of the M2 mRNA in gene expression and provides a capability for vaccine development. *Proc Natl Acad Sci USA* 1995; **92**: 11563-11567 [PMID: 8524804]
18 **Iwasaki M**, Takeda M, Shirogane Y, Nakatsu Y, Nakamura T, Yanagi Y. The matrix protein of measles virus regulates viral RNA synthesis and assembly by interacting with the nucleocapsid protein. *J Virol* 2009; **83**: 10374-10383 [PMID: 19656884 DOI: 10.1128/jvi.01056-09]
19 **Sleeman K**, Bankamp B, Hummel KB, Lo MK, Bellini WJ, Rota PA. The C, V and W proteins of Nipah virus inhibit minigenome replication. *J Gen Virol* 2008; **89**: 1300-1308 [PMID: 18420809 DOI: 10.1099/vir.0.83582-0]
20 **Flick R**, Pettersson RF. Reverse genetics system for Uukuniemi virus (Bunyaviridae): RNA polymerase I-catalyzed expression of chimeric viral RNAs. *J Virol* 2001; **75**: 1643-1655 [PMID: 11160662 DOI: 10.1128/jvi.75.4.1643-1655.2001]
21 **Fodor E**, Devenish L, Engelhardt OG, Palese P, Brownlee GG, García-Sastre A. Rescue of influenza A virus from recombinant DNA. *J Virol* 1999; **73**: 9679-9682 [PMID: 10516084]
22 **Wagner E**, Engelhardt OG, Gruber S, Haller O, Kochs G. Rescue of recombinant Thogoto virus from cloned cDNA. *J Virol* 2001; **75**: 9282-9286 [PMID: 11533190 DOI: 10.1128/jvi.75.19.9282-9286.2001]
23 **Martin A**, Staeheli P, Schneider U. RNA polymerase II-controlled expression of antigenomic RNA enhances the rescue efficacies of two different members of the Mononegavirales independently of the site of viral genome replication. *J Virol* 2006; **80**: 5708-5715 [PMID: 16731909 DOI: 10.1128/jvi.02389-05]
24 **Paule MR**, White RJ. Survey and summary: transcription by RNA polymerases I and III. *Nucleic Acids Res* 2000; **28**: 1283-1298 [PMID: 10684922 DOI: 10.1093/nar/28.6.1283]
25 **Neumann G**, Zobel A, Hobom G. RNA polymerase I-mediated expression of influenza viral RNA molecules. *Virology* 1994; **202**: 477-479 [PMID: 8009859]
26 **Hoffmann E**, Neumann G, Kawaoka Y, Hobom G, Webster RG. A DNA transfection system for generation of influenza A virus from eight plasmids. *Proc Natl Acad Sci USA* 2000; **97**: 6108-6113 [PMID: 10801978 DOI: 10.1073/pnas.100133697]
27 **Murakami S**, Horimoto T, Yamada S, Kakugawa S, Goto H, Kawaoka Y. Establishment of canine RNA polymerase I-driven reverse genetics for influenza A virus: its application for H5N1 vaccine production. *J Virol* 2008; **82**: 1605-1609 [PMID: 18045936 DOI: 10.1128/jvi.01876-07]
28 **Song MS**, Baek YH, Pascua PN, Kwon HI, Park SJ, Kim EH, Lim GJ, Choi YK. Establishment of Vero cell RNA polymerase I-driven reverse genetics for Influenza A virus and its application for pandemic (H1N1) 2009 influenza virus vaccine production. *J Gen Virol* 2013; **94**: 1230-1235 [PMID: 23486669 DOI: 10.1099/vir.0.051284-0]
29 **Marsh GA**, Virtue ER, Smith I, Todd S, Arkinstall R, Frazer L, Monaghan P, Smith GA, Broder CC, Middleton D, Wang LF. Recombinant Hendra viruses expressing a reporter gene retain pathogenicity in ferrets. *Virol J* 2013; **10**: 95 [PMID: 23521919 DOI: 10.1186/1743-422x-10-95]
30 **Yoneda M**, Guillaume V, Ikeda F, Sakuma Y, Sato H, Wild TF, Kai C. Establishment of a Nipah virus rescue system. *Proc Natl Acad Sci USA* 2006; **103**: 16508-16513 [PMID: 17053073 DOI: 10.1073/pnas.0606972103]
31 **Takeda M**, Takeuchi K, Miyajima N, Kobune F, Ami Y, Nagata N, Suzaki Y, Nagai Y, Tashiro M. Recovery of pathogenic measles virus from cloned cDNA. *J Virol* 2000; **74**: 6643-6647 [PMID: 10864679 DOI: 10.1128/jvi.74.14.6643-6647.2000]
32 **Leyrer S**, Neubert WJ, Sedlmeier R. Rapid and efficient recovery of Sendai virus from cDNA: factors influencing recombinant virus rescue. *J Virol Methods* 1998; **75**: 47-58 [PMID: 9820574 DOI: 10.1016/S0166-0934(98)00095-0]
33 **Neumann G**, Feldmann H, Watanabe S, Lukashevich I, Kawaoka Y. Reverse genetics demonstrates that proteolytic processing of the Ebola virus glycoprotein is not essential for replication in cell culture. *J Virol* 2002; **76**: 406-410 [PMID: 11739705 DOI: 10.1128/jvi.76.1.406-410.2002]
34 **Albariño CG**, Uebelhoer LS, Vincent JP, Khristova ML, Chakrabarti AK, McElroy A, Nichol ST, Towner JS. Development of a reverse genetics system to generate recombinant Marburg virus derived from a bat isolate. *Virology* 2013; **446**: 230-237 [PMID: 24074586 DOI: 10.1016/j.virol.2013.07.038]
35 **Jiang Y**, Liu H, Liu P, Kong X. Plasmids driven minigenome rescue system for Newcastle disease virus V4 strain. *Mol Biol Rep* 2009; **36**: 1909-1914 [PMID: 19011992 DOI: 10.1007/s11033-008-9398-x]
36 **Harty RN**, Brown ME, Hayes FP, Wright NT, Schnell MJ. Vaccinia virus-free recovery of vesicular stomatitis virus. *J Mol Microbiol Biotechnol* 2001; **3**: 513-517 [PMID: 11545270]
37 **Sánchez AB**, de la Torre JC. Rescue of the prototypic Arenavirus LCMV entirely from plasmid. *Virology* 2006; **350**: 370-380 [PMID: 16476461 DOI: 10.1016/j.virol.2006.01.012]
38 **Whelan SP**, Barr JN, Wertz GW. Transcription and replication of nonsegmented negative-strand RNA viruses. *Curr Top Microbiol Immunol* 2004; **283**: 61-119 [PMID: 15298168]
39 **de Wit E**, Spronken MI, Vervaet G, Rimmelzwaan GF, Osterhaus AD, Fouchier RA. A reverse-genetics system for Influenza A virus using T7 RNA polymerase. *J Gen Virol* 2007; **88**: 1281-1287 [PMID: 17374773 DOI: 10.1099/vir.0.82452-0]
40 **Collins PL**, Mink MA, Stec DS. Rescue of synthetic analogs of respiratory syncytial virus genomic RNA and effect of truncations and mutations on the expression of a foreign reporter gene. *Proc Natl Acad Sci USA* 1991; **88**: 9663-9667 [PMID: 1946383]
41 **Ghanem A**, Kern A, Conzelmann KK. Significantly improved rescue of rabies virus from cDNA plasmids. *Eur J Cell Biol* 2012; **91**: 10-16 [PMID: 21397981 DOI: 10.1016/j.ejcb.2011.01.008]
42 **Fuerst TR**, Niles EG, Studier FW, Moss B. Eukaryotic transient-expression system based on recombinant vaccinia virus that synthesizes bacteriophage T7 RNA polymerase. *Proc Natl Acad Sci USA* 1986; **83**: 8122-8126 [PMID: 3095828]
43 **Ito N**, Takayama-Ito M, Yamada K, Hosokawa J, Sugiyama M, Minamoto N. Improved recovery of rabies virus from cloned cDNA using a vaccinia virus-free reverse genetics system. *Microbiol Immunol* 2003; **47**: 613-617 [PMID: 14524622 DOI: 10.1111/j.1348-0421.2003.tb03424.x]
44 **Wyatt LS**, Moss B, Rozenblatt S. Replication-deficient vaccinia virus encoding bacteriophage T7 RNA polymerase for transient gene expression in mammalian cells. *Virology* 1995; **210**: 202-205 [PMID: 7793072 DOI: 10.1006/viro.1995.1332]
45 **Freiberg A**, Dolores LK, Enterlein S, Flick R. Establishment and characterization of plasmid-driven minigenome rescue systems for Nipah virus: RNA polymerase I- and T7-catalyzed generation of functional paramyxoviral RNA. *Virology* 2008; **370**: 33-44 [PMID: 17904180 DOI: 10.1016/j.virol.2007.08.008]
46 **Radecke F**, Spielhofer P, Schneider H, Kaelin K, Huber M, Dötsch C, Christiansen G, Billeter MA. Rescue of measles viruses from cloned DNA. *EMBO J* 1995; **14**: 5773-5784 [PMID:

8846771]
47 **Lowen AC**, Noonan C, McLees A, Elliott RM. Efficient bunyavirus rescue from cloned cDNA. *Virology* 2004; **330**: 493-500 [PMID: 15567443 DOI: 10.1016/j.virol.2004.10.009]
48 **Tong S**, Li Y, Rivailler P, Conrardy C, Castillo DA, Chen LM, Recuenco S, Ellison JA, Davis CT, York IA, Turmelle AS, Moran D, Rogers S, Shi M, Tao Y, Weil MR, Tang K, Rowe LA, Sammons S, Xu X, Frace M, Lindblade KA, Cox NJ, Anderson LJ, Rupprecht CE, Donis RO. A distinct lineage of influenza A virus from bats. *Proc Natl Acad Sci USA* 2012; **109**: 4269-4274 [PMID: 22371588]
49 **Webster RG**, Bean WJ, Gorman OT, Chambers TM, Kawaoka Y. Evolution and ecology of influenza A viruses. *Microbiol Rev* 1992; **56**: 152-179 [PMID: 1579108]
50 **Ibricevic A**, Pekosz A, Walter MJ, Newby C, Battaile JT, Brown EG, Holtzman MJ, Brody SL. Influenza virus receptor specificity and cell tropism in mouse and human airway epithelial cells. *J Virol* 2006; **80**: 7469-7480 [PMID: 16840327 DOI: 10.1128/jvi.02677-05]
51 **Thompson CI**, Barclay WS, Zambon MC, Pickles RJ. Infection of human airway epithelium by human and avian strains of influenza a virus. *J Virol* 2006; **80**: 8060-8068 [PMID: 16873262 DOI: 10.1128/jvi.00384-06]
52 **Nicholls JM**, Chan MC, Chan WY, Wong HK, Cheung CY, Kwong DL, Wong MP, Chui WH, Poon LL, Tsao SW, Guan Y, Peiris JS. Tropism of avian influenza A (H5N1) in the upper and lower respiratory tract. *Nat Med* 2007; **13**: 147-149 [PMID: 17206149 DOI: 10.1038/nm1529]
53 **Kuiken T**, Taubenberger JK. Pathology of human influenza revisited. *Vaccine* 2008; **26** Suppl 4: D59-D66 [PMID: 19230162]
54 **Neumann G**, Kawaoka Y. Reverse genetics of influenza virus. *Virology* 2001; **287**: 243-250 [PMID: 11531402 DOI: 10.1006/viro.2001.1008]
55 **Pleschka S**, Jaskunas R, Engelhardt OG, Zürcher T, Palese P, García-Sastre A. A plasmid-based reverse genetics system for influenza A virus. *J Virol* 1996; **70**: 4188-4192 [PMID: 8648766]
56 **Hoffmann E**, Zhou N, Webster RG. Eight-plasmid rescue system for Influenza A virus. *Int Congr* 2001; **1219**: 1007-1013 [DOI: 10.1016/S0531-5131(01)00375-2]
57 **Neumann G**, Fujii K, Kino Y, Kawaoka Y. An improved reverse genetics system for influenza A virus generation and its implications for vaccine production. *Proc Natl Acad Sci USA* 2005; **102**: 16825-16829 [PMID: 16267134 DOI: 10.1073/pnas.0505587102]
58 **Wiley DC**, Skehel JJ. The structure and function of the hemagglutinin membrane glycoprotein of influenza virus. *Annu Rev Biochem* 1987; **56**: 365-394 [PMID: 3304138]
59 **Han X**, Bushweller JH, Cafiso DS, Tamm LK. Membrane structure and fusion-triggering conformational change of the fusion domain from influenza hemagglutinin. *Nat Struct Biol* 2001; **8**: 715-720 [PMID: 11473264]
60 **Bertram S**, Glowacka I, Steffen I, Kühl A, Pöhlmann S. Novel insights into proteolytic cleavage of influenza virus hemagglutinin. *Rev Med Virol* 2010; **20**: 298-310 [PMID: 20629046 DOI: 10.1002/rmv.657]
61 **Garten W**, Bosch FX, Linder D, Rott R, Klenk HD. Proteolytic activation of the influenza virus hemagglutinin: The structure of the cleavage site and the enzymes involved in cleavage. *Virology* 1981; **115**: 361-374 [PMID: 7032055 DOI: 10.1016/0042-6822(81)90117-3]
62 **Böttcher E**, Matrosovich T, Beyerle M, Klenk HD, Garten W, Matrosovich M. Proteolytic activation of influenza viruses by serine proteases TMPRSS2 and HAT from human airway epithelium. *J Virol* 2006; **80**: 9896-9898 [PMID: 16973594 DOI: 10.1128/JVI.01118-06]
63 **Zhirnov OP**, Ikizler MR, Wright PF. Cleavage of influenza a virus hemagglutinin in human respiratory epithelium is cell associated and sensitive to exogenous antiproteases. *J Virol* 2002; **76**: 8682-8689 [PMID: 12163588 DOI: 10.1128/JVI.76.17.8682-8689.2002]
64 **Horimoto T**, Rivera E, Pearson J, Senne D, Krauss S, Kawaoka Y, Webster RG. Origin and molecular changes associated with emergence of a highly pathogenic H5N2 influenza virus in Mexico. *Virology* 1995; **213**: 223-230 [PMID: 7483266 DOI: 10.1006/viro.1995.1562]
65 **Senne DA**, Panigrahy B, Kawaoka Y, Pearson JE, Süss J, Lipkind M, Kida H, Webster RG. Survey of the hemagglutinin (HA) cleavage site sequence of H5 and H7 avian influenza viruses: amino acid sequence at the HA cleavage site as a marker of pathogenicity potential. *Avian Dis* 1996; **40**: 425-437 [PMID: 8790895 DOI: 10.2307/1592241]
66 **Horimoto T**, Kawaoka Y. Reverse genetics provides direct evidence for a correlation of hemagglutinin cleavability and virulence of an avian influenza A virus. *J Virol* 1994; **68**: 3120-3128 [PMID: 8151777]
67 **Hatta M**, Gao P, Halfmann P, Kawaoka Y. Molecular basis for high virulence of Hong Kong H5N1 influenza A viruses. *Science* 2001; **293**: 1840-1842 [PMID: 11546875 DOI: 10.1126/science.1062882]
68 **Schrauwen EJ**, Herfst S, Leijten LM, van Run P, Bestebroer TM, Linster M, Bodewes R, Kreijtz JH, Rimmelzwaan GF, Osterhaus AD, Fouchier RA, Kuiken T, van Riel D. The multibasic cleavage site in H5N1 virus is critical for systemic spread along the olfactory and hematogenous routes in ferrets. *J Virol* 2012; **86**: 3975-3984 [PMID: 22278228 DOI: 10.1128/JVI.06828-11]
69 **Suguitan AL**, Matsuoka Y, Lau YF, Santos CP, Vogel L, Cheng LI, Orandle M, Subbarao K. The multibasic cleavage site of the hemagglutinin of highly pathogenic A/Vietnam/1203/2004 (H5N1) avian influenza virus acts as a virulence factor in a host-specific manner in mammals. *J Virol* 2012; **86**: 2706-2714 [PMID: 22205751 DOI: 10.1128/JVI.05546-11]
70 **Gao R**, Dong L, Dong J, Wen L, Zhang Y, Yu H, Feng Z, Chen M, Tan Y, Mo Z, Liu H, Fan Y, Li K, Li CK, Li D, Yang W, Shu Y. A systematic molecular pathology study of a laboratory confirmed H5N1 human case. *PLoS One* 2010; **5**: e13315 [PMID: 20976271 DOI: 10.1371/journal.pone.0013315]
71 **de Jong MD**, Simmons CP, Thanh TT, Hien VM, Smith GJ, Chau TN, Hoang DM, Chau NV, Khanh TH, Dong VC, Qui PT, Cam BV, Ha do Q, Guan Y, Peiris JS, Chinh NT, Hien TT, Farrar J. Fatal outcome of human influenza A (H5N1) is associated with high viral load and hypercytokinemia. *Nat Med* 2006; **12**: 1203-1207 [PMID: 16964257 DOI: 10.1038/nm1477]
72 **Böttcher-Friebertshäuser E**, Lu Y, Meyer D, Sielaff F, Steinmetzer T, Klenk HD, Garten W. Hemagglutinin activating host cell proteases provide promising drug targets for the treatment of influenza A and B virus infections. *Vaccine* 2012; **30**: 7374-7380 [PMID: 23072892 DOI: 10.1016/j.vaccine.2012.10.001]
73 **Feldmann H**, Jones S, Klenk HD, Schnittler HJ. Ebola virus: from discovery to vaccine. *Nat Rev Immunol* 2003; **3**: 677-685 [PMID: 12974482]
74 **Leroy EM**, Epelboin A, Mondonge V, Pourrut X, Gonzalez JP, Muyembe-Tamfum JJ, Formenty P. Human Ebola outbreak resulting from direct exposure to fruit bats in Luebo, Democratic Republic of Congo, 2007. *Vector Borne Zoonotic Dis* 2009; **9**: 723-728 [PMID: 19323614 DOI: 10.1089/vbz.2008.0167]
75 **Leroy EM**, Kumulungui B, Pourrut X, Rouquet P, Hassanin A, Yaba P, Délicat A, Paweska JT, Gonzalez JP, Swanepoel R. Fruit bats as reservoirs of Ebola virus. *Nature* 2005; **438**: 575-576 [PMID: 16319873 DOI: 10.1038/438575a]
76 **Brown DW**. Threat to Humans from Virus Infections of Non-human Primates. *Rev Med Virol* 1997; **7**: 239-246 [PMID: 10398488 DOI: 10.1002/(sici)1099-1654(199712)7: 4<239: : aid-rmv210>3.0.co; 2-q]

77 **Baron RC**, McCormick JB, Zubeir OA. Ebola virus disease in southern Sudan: hospital dissemination and intrafamilial spread. *Bull World Health Organ* 1983; **61**: 997-1003 [PMID: 6370486]

78 **Kinsman J**. "A time of fear": local, national, and international responses to a large Ebola outbreak in Uganda. *Global Health* 2012; **8**: 15 [PMID: 22695277 DOI: 10.1186/1744-8603-8-15]

79 **MacNeil A**, Farnon EC, Wamala J, Okware S, Cannon DL, Reed Z, Towner JS, Tappero JW, Lutwama J, Downing R, Nichol ST, Ksiazek TG, Rollin PE. Proportion of deaths and clinical features in Bundibugyo Ebola virus infection, Uganda. *Emerg Infect Dis* 2010; **16**: 1969-1972 [PMID: 21122234 DOI: 10.3201/eid1612.100627]

80 **Mahanty S**, Bray M. Pathogenesis of filoviral haemorrhagic fevers. *Lancet Infect Dis* 2004; **4**: 487-498 [PMID: 15288821 DOI: 10.1016/S1473-3099(04)01103-X]

81 **Towner JS**, Sealy TK, Khristova ML, Albariño CG, Conlan S, Reeder SA, Quan PL, Lipkin WI, Downing R, Tappero JW, Okware S, Lutwama J, Bakamutumaho B, Kayiwa J, Comer JA, Rollin PE, Ksiazek TG, Nichol ST. Newly discovered ebola virus associated with hemorrhagic fever outbreak in Uganda. *PLoS Pathog* 2008; **4**: e1000212 [PMID: 19023410 DOI: 10.1371/journal.ppat.1000212]

82 Filovirus infections among persons with occupational exposure to nonhuman primates. Update. *Wkly Epidemiol Rec* 1990; **65**: 185-186 [PMID: 2386703]

83 **Miranda ME**, Miranda NL. Reston ebolavirus in humans and animals in the Philippines: a review. *J Infect Dis* 2011; **204** Suppl 3: S757-S760 [PMID: 21987747 DOI: 10.1093/infdis/jir296]

84 **Theriault S**, Groseth A, Artsob H, Feldmann H. The role of reverse genetics systems in determining filovirus pathogenicity. *Arch Virol Suppl* 2005; **(19)**: 157-177 [PMID: 16355872]

85 **Takada A**, Robison C, Goto H, Sanchez A, Murti KG, Whitt MA, Kawaoka Y. A system for functional analysis of Ebola virus glycoprotein. *Proc Natl Acad Sci USA* 1997; **94**: 14764-14769 [PMID: 9405687 DOI: 10.1007/3-211-29981-5_13]

86 **Göringer H**. RNA editing. Heidelberg: Springer, 2008 [DOI: 10.1007/978-3-540-73787-2]

87 **Volchkov VE**, Volchkova VA, Muhlberger E, Kolesnikova LV, Weik M, Dolnik O, Klenk HD. Recovery of infectious Ebola virus from complementary DNA: RNA editing of the GP gene and viral cytotoxicity. *Science* 2001; **291**: 1965-1969 [PMID: 11239157 DOI: 10.1126/science.1057269]

88 **Feldmann H**, Volchkov VE, Volchkova VA, Ströher U, Klenk HD. Biosynthesis and role of filoviral glycoproteins. *J Gen Virol* 2001; **82**: 2839-2848 [PMID: 11714958]

89 **Yang ZY**, Duckers HJ, Sullivan NJ, Sanchez A, Nabel EG, Nabel GJ. Identification of the Ebola virus glycoprotein as the main viral determinant of vascular cell cytotoxicity and injury. *Nat Med* 2000; **6**: 886-889 [PMID: 10932225]

90 **Groseth A**, Marzi A, Hoenen T, Herwig A, Gardner D, Becker S, Ebihara H, Feldmann H. The Ebola virus glycoprotein contributes to but is not sufficient for virulence in vivo. *PLoS Pathog* 2012; **8**: e1002847 [PMID: 22876185 DOI: 10.1371/journal.ppat.1002847]

91 **Ebihara H**, Takada A, Kobasa D, Jones S, Neumann G, Theriault S, Bray M, Feldmann H, Kawaoka Y. Molecular determinants of Ebola virus virulence in mice. *PLoS Pathog* 2006; **2**: e73 [PMID: 16848640 DOI: 10.1371/journal.ppat.0020073]

92 **Hartman AL**, Dover JE, Towner JS, Nichol ST. Reverse genetic generation of recombinant Zaire Ebola viruses containing disrupted IRF-3 inhibitory domains results in attenuated virus growth in vitro and higher levels of IRF-3 activation without inhibiting viral transcription or replication. *J Virol* 2006; **80**: 6430-6440 [PMID: 16775331 DOI: 10.1128/jvi.00044-06]

93 **Hartman AL**, Ling L, Nichol ST, Hibberd ML. Whole-genome expression profiling reveals that inhibition of host innate immune response pathways by Ebola virus can be reversed by a single amino acid change in the VP35 protein. *J Virol* 2008; **82**: 5348-5358 [PMID: 18353943 DOI: 10.1128/jvi.00215-08]

94 **Blackwood JC**, Streicker DG, Altizer S, Rohani P. Resolving the roles of immunity, pathogenesis, and immigration for rabies persistence in vampire bats. *Proc Natl Acad Sci USA* 2013; **110**: 20837-20842 [PMID: 24297874 DOI: 10.1073/pnas.1308817110]

95 **Real LA**, Henderson JC, Biek R, Snaman J, Jack TL, Childs JE, Stahl E, Waller L, Tinline R, Nadin-Davis S. Unifying the spatial population dynamics and molecular evolution of epidemic rabies virus. *Proc Natl Acad Sci USA* 2005; **102**: 12107-12111 [PMID: 16103358 DOI: 10.1073/pnas.0500057102]

96 **Knobel DL**, Cleaveland S, Coleman PG, Fèvre EM, Meltzer MI, Miranda ME, Shaw A, Zinsstag J, Meslin FX. Re-evaluating the burden of rabies in Africa and Asia. *Bull World Health Organ* 2005; **83**: 360-368 [PMID: 15976877]

97 **Chomel BB**, Belotto A, Meslin FX. Wildlife, exotic pets, and emerging zoonoses. *Emerg Infect Dis* 2007; **13**: 6-11 [PMID: 17370509]

98 **Gautret P**, Blanton J, Dacheux L, Ribadeau-Dumas F, Brouqui P, Parola P, Esposito DH, Bourhy H. Rabies in non-human primates and potential for transmission to humans: a literature review and examination of selected French national data. *PLoS Negl Trop Dis* 2014; **8**: e2863 [PMID: 24831694 DOI: 10.1371/journal.pntd.0002863]

99 **Piyaphanee W**, Shantavasinkul P, Phumratanaprapin W, Udomchaisakul P, Wichianprasat P, Benjavongkulchai M, Ponam T, Tantawichian T. Rabies exposure risk among foreign backpackers in Southeast Asia. *Am J Trop Med Hyg* 2010; **82**: 1168-1171 [PMID: 20519619 DOI: 10.4269/ajtmh.2010.09-0699]

100 **Mazarakis ND**, Azzouz M, Rohll JB, Ellard FM, Wilkes FJ, Olsen AL, Carter EE, Barber RD, Baban DF, Kingsman SM, Kingsman AJ, O'Malley K, Mitrophanous KA. Rabies virus glycoprotein pseudotyping of lentiviral vectors enables retrograde axonal transport and access to the nervous system after peripheral delivery. *Hum Mol Genet* 2001; **10**: 2109-2121 [PMID: 11590128 DOI: 10.1093/hmg/10.19.2109]

101 **Pulmanausahakul R**, Li J, Schnell MJ, Dietzschold B. The glycoprotein and the matrix protein of rabies virus affect pathogenicity by regulating viral replication and facilitating cell-to-cell spread. *J Virol* 2008; **82**: 2330-2338 [PMID: 18094173 DOI: 10.1128/jvi.02327-07]

102 **Wiltzer L**, Okada K, Yamaoka S, Larrous F, Kuusisto HV, Sugiyama M, Blondel D, Bourhy H, Jans DA, Ito N, Moseley GW. Interaction of rabies virus P-protein with STAT proteins is critical to lethal rabies disease. *J Infect Dis* 2014; **209**: 1744-1753 [PMID: 24367042 DOI: 10.1093/infdis/jit829]

103 **Manning SE**, Rupprecht CE, Fishbein D, Hanlon CA, Lumlertdacha B, Guerra M, Meltzer MI, Dhankhar P, Vaidya SA, Jenkins SR, Sun B, Hull HF. Human rabies prevention--United States, 2008: recommendations of the Advisory Committee on Immunization Practices. *MMWR Recomm Rep* 2008; **57**: 1-28 [PMID: 18496505]

104 **WHO**. Rabies vaccines: WHO position paper--recommendations. *Vaccine* 2010; **28**: 7140-7142 [PMID: 20831913]

105 **Faber M**, Pulmanausahakul R, Hodawadekar SS, Spitsin S, McGettigan JP, Schnell MJ, Dietzschold B. Overexpression of the rabies virus glycoprotein results in enhancement of apoptosis and antiviral immune response. *J Virol* 2002; **76**: 3374-3381 [PMID: 11884563 DOI: 10.1128/jvi.76.7.3374-3381.2002]

106 **Cenna J**, Hunter M, Tan GS, Papaneri AB, Ribka EP, Schnell MJ, Marx PA, McGettigan JP. Replication-deficient rabies virus-based vaccines are safe and immunogenic in mice and nonhuman primates. *J Infect Dis* 2009; **200**: 1251-1260 [PMID: 19764884 DOI: 10.1086/605949]

107 **Blaney JE**, Wirblich C, Papaneri AB, Johnson RF, Myers CJ, Juelich TL, Holbrook MR, Freiberg AN, Bernbaum

JG, Jahrling PB, Paragas J, Schnell MJ. Inactivated or live-attenuated bivalent vaccines that confer protection against rabies and Ebola viruses. *J Virol* 2011; **85**: 10605-10616 [PMID: 21849459 DOI: 10.1128/jvi.00558-11]

108 **Halpin K**, Young PL, Field HE, Mackenzie JS. Isolation of Hendra virus from pteropid bats: a natural reservoir of Hendra virus. *J Gen Virol* 2000; **81**: 1927-1932 [PMID: 10900029]

109 **Dhondt KP,** Horvat B. Hendra and Nipah viruses. In: Munir M. Mononegaviruses of veterinary importance. Volume 1, Pathobiology and molecular diagnosis. Oxfordshire, UK: CAB International, 2013: 38

110 **Chua KB**. Nipah virus outbreak in Malaysia. *J Clin Virol* 2003; **26**: 265-275 [PMID: 12637075 DOI: 10.1016/S1386-6532(02)00268-8]

111 **Rahman MA**, Hossain MJ, Sultana S, Homaira N, Khan SU, Rahman M, Gurley ES, Rollin PE, Lo MK, Comer JA, Lowe L, Rota PA, Ksiazek TG, Kenah E, Sharker Y, Luby SP. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector Borne Zoonotic Dis* 2012; **12**: 65-72 [PMID: 21923274 DOI: 10.1089/vbz.2011.0656]

112 **Sazzad HM**, Hossain MJ, Gurley ES, Ameen KM, Parveen S, Islam MS, Faruque LI, Podder G, Banu SS, Lo MK, Rollin PE, Rota PA, Daszak P, Rahman M, Luby SP. Nipah virus infection outbreak with nosocomial and corpse-to-human transmission, Bangladesh. *Emerg Infect Dis* 2013; **19**: 210-217 [PMID: 23347678 DOI: 10.3201/eid1902.120971]

113 **Wong KT**, Shieh WJ, Kumar S, Norain K, Abdullah W, Guarner J, Goldsmith CS, Chua KB, Lam SK, Tan CT, Goh KJ, Chong HT, Jusoh R, Rollin PE, Ksiazek TG, Zaki SR. Nipah virus infection: pathology and pathogenesis of an emerging paramyxoviral zoonosis. *Am J Pathol* 2002; **161**: 2153-2167 [PMID: 12466131 DOI: 10.1016/S0002-9440(10)64493-8]

114 **Eaton BT**, Broder CC, Middleton D, Wang LF. Hendra and Nipah viruses: different and dangerous. *Nat Rev Microbiol* 2006; **4**: 23-35 [PMID: 16357858 DOI: 10.1038/nrmicro1323]

115 **O'Sullivan JD**, Allworth AM, Paterson DL, Snow TM, Boots R, Gleeson LJ, Gould AR, Hyatt AD, Bradfield J. Fatal encephalitis due to novel paramyxovirus transmitted from horses. *Lancet* 1997; **349**: 93-95 [PMID: 8996421 DOI: 10.1016/S0140-6736(96)06162-4]

116 **Murray K**, Selleck P, Hooper P, Hyatt A, Gould A, Gleeson L, Westbury H, Hiley L, Selvey L, Rodwell B. A morbillivirus that caused fatal disease in horses and humans. *Science* 1995; **268**: 94-97 [PMID: 7701348 DOI: 10.1126/science.7701348]

117 **Rockx B**, Winegar R, Freiberg AN. Recent progress in henipavirus research: molecular biology, genetic diversity, animal models. *Antiviral Res* 2012; **95**: 135-149 [PMID: 22643730 DOI: 10.1016/j.antiviral.2012.05.008]

118 **Rota PA**, Lo MK. Molecular virology of the henipaviruses. *Curr Top Microbiol Immunol* 2012; **359**: 41-58 [PMID: 22552699 DOI: 10.1007/82_2012_211]

119 **Kulkarni S**, Volchkova V, Basler CF, Palese P, Volchkov VE, Shaw ML. Nipah virus edits its P gene at high frequency to express the V and W proteins. *J Virol* 2009; **83**: 3982-3987 [PMID: 19211754 DOI: 10.1128/jvi.02599-08]

120 **Childs KS**, Andrejeva J, Randall RE, Goodbourn S. Mechanism of mda-5 Inhibition by paramyxovirus V proteins. *J Virol* 2009; **83**: 1465-1473 [PMID: 19019954 DOI: 10.1128/jvi.01768-08]

121 **Andrejeva J**, Childs KS, Young DF, Carlos TS, Stock N, Goodbourn S, Randall RE. The V proteins of paramyxoviruses bind the IFN-inducible RNA helicase, mda-5, and inhibit its activation of the IFN-beta promoter. *Proc Natl Acad Sci USA* 2004; **101**: 17264-17269 [PMID: 15563593 DOI: 10.1073/pnas.0407639101]

122 **Shaw ML**. Henipaviruses employ a multifaceted approach to evade the antiviral interferon response. *Viruses* 2009; **1**: 1190-1203 [PMID: 21994589 DOI: 10.3390/v1031190]

123 **Yoneda M**, Guillaume V, Sato H, Fujita K, Georges-Courbot MC, Ikeda F, Omi M, Muto-Terao Y, Wild TF, Kai C. The nonstructural proteins of Nipah virus play a key role in pathogenicity in experimentally infected animals. *PLoS One* 2010; **5**: e12709 [PMID: 20856799 DOI: 10.1371/journal.pone.0012709]

124 **Mathieu C**, Guillaume V, Volchkova VA, Pohl C, Jacquot F, Looi RY, Wong KT, Legras-Lachuer C, Volchkov VE, Lachuer J, Horvat B. Nonstructural Nipah virus C protein regulates both the early host proinflammatory response and viral virulence. *J Virol* 2012; **86**: 10766-10775 [PMID: 22837207 DOI: 10.1128/jvi.01203-12]

125 **Lauring AS**, Andino R. Quasispecies theory and the behavior of RNA viruses. *PLoS Pathog* 2010; **6**: e1001005 [PMID: 20661479 DOI: 10.1371/journal.ppat.1001005]

126 **Gutiérrez RA**, Viari A, Godelle B, Frutos R, Buchy P. Biased mutational pattern and quasispecies hypothesis in H5N1 virus. *Infect Genet Evol* 2013; **15**: 69-76 [PMID: 22063822 DOI: 10.1016/j.meegid.2011.10.019]

127 **Biebricher CK**, Eigen M. The error threshold. *Virus Res* 2005; **107**: 117-127 [PMID: 15649558 DOI: 10.1016/j.virusres.2004.11.002]

128 **Tsai L**, Tasovski I, Leda AR, Chin MP, Cheng-Mayer C. The number and genetic relatedness of transmitted/founder virus impact clinical outcome in vaginal R5 SHIVSF162P3N infection. *Retrovirology* 2014; **11**: 22 [PMID: 24612462 DOI: 10.1186/1742-4690-11-22]

129 **Vignuzzi M**, Stone JK, Arnold JJ, Cameron CE, Andino R. Quasispecies diversity determines pathogenesis through cooperative interactions in a viral population. *Nature* 2006; **439**: 344-348 [PMID: 16327776 DOI: 10.1038/nature04388]

130 **Gibson DG**, Benders GA, Andrews-Pfannkoch C, Denisova EA, Baden-Tillson H, Zaveri J, Stockwell TB, Brownley A, Thomas DW, Algire MA, Merryman C, Young L, Noskov VN, Glass JI, Venter JC, Hutchison CA, Smith HO. Complete chemical synthesis, assembly, and cloning of a Mycoplasma genitalium genome. *Science* 2008; **319**: 1215-1220 [PMID: 18218864 DOI: 10.1126/science.1151721]

131 **Lauring AS**, Andino R. Exploring the fitness landscape of an RNA virus by using a universal barcode microarray. *J Virol* 2011; **85**: 3780-3791 [PMID: 21289109 DOI: 10.1128/jvi.02217-10]

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REVIEW

Subversion of cellular stress responses by poxviruses

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Abstract

Cellular stress responses are powerful mechanisms that prevent and cope with the accumulation of macromolecular damage in the cells and also boost host defenses against pathogens. Cells can initiate either protective or destructive stress responses depending, to a large extent, on the nature and duration of the stressing stimulus as well as the cell type. The productive replication of a virus within a given cell places inordinate stress on the metabolism machinery of the host and, to assure the continuity of its replication, many viruses have developed ways to modulate the cell stress responses. Poxviruses are among the viruses that have evolved a large number of strategies to manipulate host stress responses in order to control cell fate and enhance their replicative success. Remarkably, nearly every step of the stress responses that is mounted during infection can be targeted by virally encoded functions. The fine-tuned interactions between poxviruses and the host stress responses has aided virologists to understand specific aspects of viral replication; has helped cell biologists to evaluate the role of stress signaling in the uninfected cell; and has tipped immunologists on how these signals contribute to alert the cells against pathogen invasion and boost subsequent immune responses. This review discusses the diverse strategies that poxviruses use to subvert host cell stress responses.

Key words: Poxvirus; Cell stress response; Heat shock response; Chaperones; Unfolded protein response; Host translational control; Hypoxia; Oxidative stress; DNA damage

Core tip: Poxviruses are known to encode a plethora of proteins that interact with cell biology processes in order to achieve replicative success. In this article, we review how poxviruses cope with cellular stress signals that are usually triggered upon infection to tentatively block virus replication. The understanding of mechanisms by which poxviruses and other complex viruses interfere with stress responses can further illuminate the web of pathways regulating cell homeostasis, as well as how viruses intertwine their own biochemical needs into this intricate scenario.

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INTRODUCTION

The Poxviridae family is taxonomically divided into two subfamilies of double-stranded DNA (dsDNA) viruses that are able to infect insects (Entomopoxvirinae) and a wide spectrum of vertebrate hosts (Chordopoxvirinae). The Chordopoxvirinae subfamily currently contains ten genera (Avipoxvirus, Capripoxvirus, Cervidpoxvirus, Crocodylidpoxvirus, Leporipoxvirus, Molluscipoxvirus, Orthopoxvirus, Parapoxvirus, Suinopoxvirus, Yatapoxvirus) and one unassigned species (Squirrelpox virus), whereas the Entomopoxvirinae subfamily comprises three genera

(Alphaentomopoxvirus, Betaentomopoxvirus, Gammaentomopoxvirus) and two unclassified species (Diachasmimorpha entomopoxvirus and Melanoplus sanguinipes entomopoxvirus "O")[1]. Members of the Poxviridae family are large viruses (approximately 350 nm × 250 nm × 200 nm) with a linear genome ranging from 130 to 300 kb, each often encoding approximately 200 proteins. Virions are brick-shaped, multi-enveloped particles and, unlike other DNA viruses, replicate exclusively in the cytoplasm of the infected host cell. Most poxviral biosynthetic pathways occur in distinct sites of the cytoplasm called viral factories: large masses of electron dense material, the viroplasm, that are frequently surrounded by membranes from the endoplasmic reticulum (ER) and/or membranes from the ER-Golgi intermediate compartments[2-6].

Different viruses have evolved two very distinct general strategies to compete with the host cell for biochemical resources and successfully replicate within them. One such strategy is a "hit and run" type of approach, in which viruses rapidly replicate and generate a progeny that spreads quickly to other cells. In order to be effective, these viruses have invested in replication speed by keeping small genomes which code for few essential proteins - the faster they replicate, the more efficiently they can escape antiviral responses by the host. A second strategy, however, is based on a "stay and fight" approach. Viruses that adopted this strategy tend to endure within the host cell and, therefore, may be susceptible to antiviral responses that are gradually elicited against them. Thus, in order to achieve replicative success, these viruses have to cope with the host attempts to get rid of them and, as a way to counteract antiviral responses, many evolved processes to either block or delay such responses. Because most viral strategies to evade host responses are based in virus-coded proteins, this led inevitably to an increase in genome sizes. There are obvious exceptions to this rather simplistic classification of virus replication strategies, as in the case of hepadnaviruses (like hepatitis B virus) for instance. Nonetheless, most viruses can still fit one of the two aforementioned models. Poxviruses are one of the best examples of viruses that have developed ways to either counteract host strategies to hamper viral replication or boost their biosynthetic pathways to the detriment of the host's. Indeed, most poxviruses (especially chordopoxviruses) spare up to 50% of their genomes to code for immune evasion-related and host-interaction genes[7].

As soon as these viruses enter the host cell, they set in motion a number of biochemical strategies to usurp cellular resources. One such strategy is to hijack the host translation apparatus to selectively produce large quantities of viral proteins. To this end, poxviruses produce proteins that are able to cleave host messenger RNAs (mRNAs)[4,8-10] early in infection, shutting down the host protein synthesis almost completely during the first hours of the viral cycle[11]. Furthermore, viruses are devoid of molecular chaperones, such as heat shock proteins (HSPs) with few exceptions and rely almost completely on chaperones of the host to adequately process viral proteins, avoiding misfolding or aggregation[12-14]. In parallel, viral double-stranded RNA intermediates, DNA and proteins are sensed by pattern recognition receptors in the cell, leading to the generation of innate immune responses potentially able to control the viral infection[15,16].

All the above mentioned virus-driven interferences within the cell may lead to the transduction of cell stress signals and consequent cell stress responses. The cell may respond to stress in a variety of ways, including the activation of pathways that promote survival or the elimination of damaged cells through programmed cell death (apoptosis, necrosis and/or autophagy). There is a multitude of pathways that may be elicited upon different types of stress and the resulting signal transduction cascades are often shared by other cell processes, such as the activation of innate immunity, cell cycling and so on. Nevertheless, the most common stress responses include those elicited against heat shock, ER stress (the unfolded protein response, UPR), DNA damage, hypoxia and oxidative stress. Some of these responses may limit or inhibit viral replication and/or induce cell death and others can promote cell survival and restore homeostasis. To cope with stress responses, poxviruses have evolved complex molecular strategies to counteract innate host cell defense signaling pathways while facilitating biological events that promote adaptation and survival of the host cell, all essential to a productive infection. This review summarizes the main cellular stress responses used or subverted by poxviruses to ensure completion of viral life cycle.

HEAT SHOCK RESPONSE

In the early 1960s, the discovery of the heat shock response (HSR) led to the elucidation of some aspects of the cell stress responses and the discovery of heat shock genes[17] and proteins (HSPs)[18,19]. Many HSPs are constitutively present in cells while some are expressed only after stress. HSPs and other molecular chaperones (*e.g.*, co-chaperones and folding enzymes) are active in a myriad of biological essential processes that include: (1) the normal folding of polypeptides; (2) assisting misfolded proteins to attain or regain their native states; (3) regulation of protein degradation; and (4) translocation of proteins across membranes to different cellular compartments[20,21]. Some of these proteins are conserved in all three superkingdoms and are encoded by genes that contain cis-acting regulatory sequences, termed heat shock elements (HSE), which are regulated by heat shock transcription factors (HSFs)[22,23]. Upon stress, one of the main regulators of the HSR, the HSF1, undergoes trimerization and subsequent translocation into the nucleus where these complexes bind to the HSE[23] (Figure 1). HSF1 is regulated by post-translational modifications such as phosphorylation, acetylation[24], sumoylation[25,26] and interactions with other proteins. HSF1 is constitutively expressed and is neither a stress-inducible protein nor is its expression correlated with the

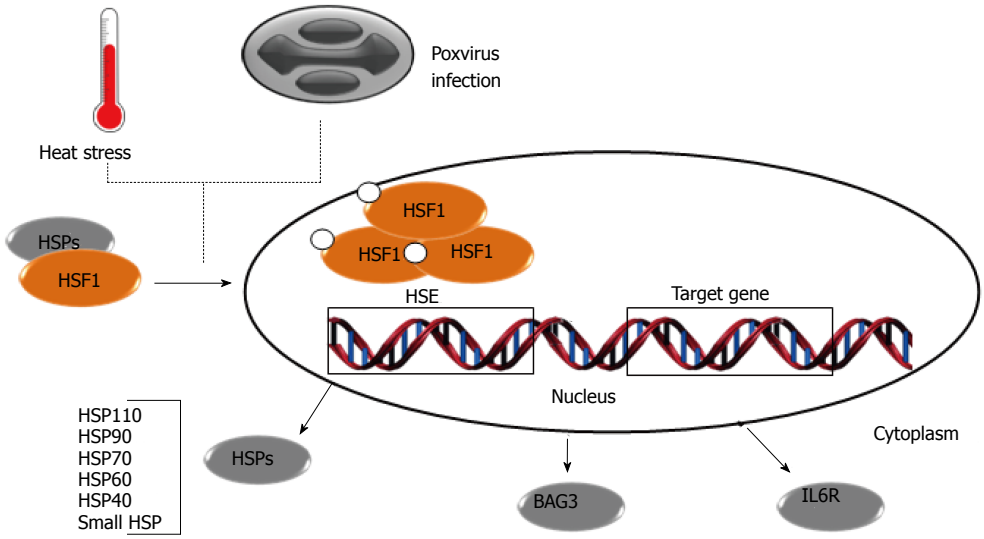

Figure 1 Heat shock responses induced by poxviruses. Under normal conditions, HSFs interact with HSPs or exist as a monomer in the cytosol. Upon exposure to stress conditions such as heat shock, oxidative stress or poxvirus infection, HSF1 undergoes post-translational modifications, such as phosphorylation, trimerizes and migrates to the nucleus. In the nucleus, HSF1 trimer binds to the HSE, leading to induction of all classes of HSPs and other chaperones. HSE: Heat shock elements; HSFs: Heat shock transcription factors; HSPs: Heat shock proteins.

expression of heat shock genes[27] (Figure 1).

Recent studies, using different genome-scale approaches to identify host proteins used by poxviruses during infection, revealed that HSF1 is a crucial transcription factor for virus replication and some targets of HSF1 are induced upon infection[28]. At the early stages of poxvirus infections, a decrease in HSF1 mRNA synthesis is observed; however, this does not seem to affect the protein levels as its half-life is quite long. As the viral lifecycle progresses, an increase in HSF1 mRNA levels can be detected, although this is not followed by augmentation in this protein contents within the cell[29]. Infections by some poxviruses result in the phosphorylation of HSF1 and its translocations to nucleus, where they bind to HSE[28,29]. Several HSF1-regulated genes are upregulated during infection, including genes coding for the molecular chaperones *BAG3*, *STIP1,* all classes of HSPs (HSP10, HSP20, HSP40, HSP60, HSP70, HSP90 and HSP105/110) and other important proteins like *IL6R,* which has a role in cell growth and differentiation[8,28,30] (Figure 1).

The first observation of the interaction between poxvirus and HSPs was made by Jindal *et al*[10] (1992) who also showed that the infection led to a small increase in HSP90 and HSP60 mRNA contents and to a substantial increase in the HSP70 mRNA levels, suggesting that these proteins may play some role in viral protein folding. Opposed to this view, subsequent studies revealed that the overexpression of the 72 kDa HSP, the major inducible cytoplasmic HSP, did not affect virus replication[31,32]. Furthermore, during poxvirus infections, HSP70 accumulates predominantly in the nucleus where these proteins interact with poly (ADP-ribose) polymerase 1, PARP1 and XRCC1 and prevent single-stranded DNA break (SSB)[29,33]. Globally, these observations suggest that HSP70s are important for cell survival and death prevention but may have a lesser impact in the proper folding of poxviral proteins.

So far, the most likely HSP to have a role in the poxvirus life cycle is HSP90. This chaperone is the most abundant HSP in unstressed cells and many of its targets are either kinases or transcription factors such as Akt and HSF1, respectively[34,35]. The inhibition of HSP90 function during infection by the use of geldanamycin, a drug that blocks the ATPase activity of that chaperone, impairs viral multiplication by delaying viral DNA replication and intermediate transcription, and also by reducing expression of late genes[36].

It has been shown that HSP90 interacts directly with the 4a core protein (encoded by *A10L* orthologous genes), implicating this chaperone in conformational maturation of the poxvirus capsid. Nonetheless, HSP90 does not colocalize with capsid proteins at later stages of infection, suggesting a transient role for HSP90 in virion morphogenesis[36]. Other host chaperones (*e.g.*, cyclophilin A and Hsc71) are found to be associated with intracellular mature virions (IMV) but the importance of these proteins in such a context needs be further investigated[37,38].

UNFOLDED PROTEIN RESPONSE

The endoplasmic reticulum (ER) is a multifunctional organelle that controls several critical aspects of cellular processes: it ensures the correct structure of most proteins; plays a key role in the synthesis of lipids and sterols; and helps in the maintenance of intracellular calcium levels and many other functions[39]. The protein homeo-

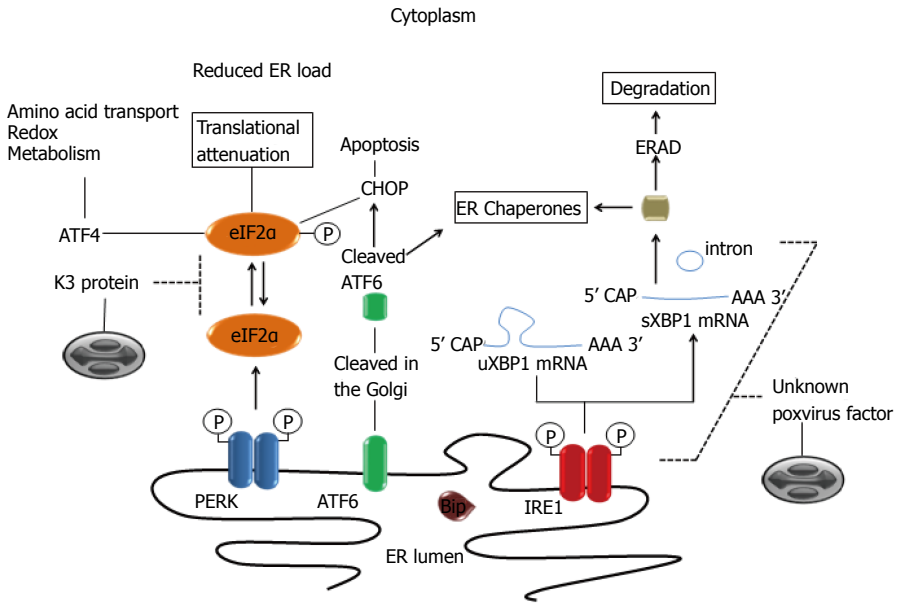

Figure 2 Modulation of mammalian unfolded protein response pathways by poxviruses. ER stress is sensed by three ER-membrane bound sensors [PERK, ATF6 and Inositol-requiring protein 1 (IRE1)]. Under conditions of ER stress, unfolded proteins accumulate in the ER lumen causing the initiation of a coordinated signaling pathway, the unfolded protein response (UPR), to restore ER homeostasis. ATF6 traffics to the Golgi, where site-specific proteases (S1, S2) cleave it into an active transcription factor. Protein kinase PERK oligomerizes and is activated *via* trans-autophosphorylation. IRE1 is both a kinase and an endonuclease that splices 26bp from the X-box binding protein 1 (XBP1) mRNA. XBP1 is a transcription factor that regulates positively the expression of many essential UPR genes involved in folding and quality control of proteins. Poxviruses evade XBP1 splicing by an unknown mechanism. Activated PERK phosphorylates eIF2a, resulting in global translational attenuation. However, some mRNA such as ATF4 gains a selective advantage for translation *via* phosphorylated eIF2. ATF4 in turn contributes to cytoprotection. Expression of other UPR gene targets (*e.g.*, CHOP) may result in cell death. Poxviruses K3L orthologous genes code for proteins that bind to PERK as a pseudo-substrate and thus inhibit eIF2a phosphorylation. ER: Endoplasmic reticulum; PERK: Protein kinase RNA-like ER kinase; eIF2a: Elongation initiation factor 2a; ATF: Activating transcription factor.

stasis (proteostasis) surveillance in the ER is mediated by specific pathways generally called unfolded protein response (UPR), which is activated when the intrinsic protein folding capacity of the organelle is overwhelmed by a large input of unfolded proteins into the ER[40,41]. Such imbalance activates three signaling pathways through ER-resident transmembrane proteins [inositol-requiring protein 1 (IRE1), activating transcription factor 6 (ATF6) and protein kinase RNA-like ER kinase (PERK)], resulting either in recovery of proteostasis or in cell death[42,43] (Figure 2). In resting cells, these molecular sensors are maintained in inactive states through interactions with the major and most abundant ER-resident chaperone, the binding immunoglobulin protein (BiP) (Figure 2), also known as glucose regulated protein of 78 kDa (GRP78), encoded by the *HSPA5* gene[44,45].

Because poxviruses replicate in close association with the ER, using components of this organelle to its own benefit, it was suggested that they might trigger ER stress and activate UPR signaling[46]. Indeed, many structural Vaccinia virus (the prototypic member of the family) proteins are known to closely interact with membranes of the ER during the formation of crescent membranes and immature virions[47-49]. Yet, no activation of IRE1-dependent stress pathways is usually detected[50] and how poxviruses evade and/or subvert this UPR signaling is still not known. During ER stress, IRE1 undergoes dissociation from BiP and BAX inhibitor 1[51,52], triggering its dimerization and the activation of its endonuclease activity[53-55]. The IRE1 nuclease domain has homology to RNase L and its activation causes splicing of a residual intron (26nt) in the XBP1 mRNA, resulting in a more stable and active form of the XBP1 protein (HAC1 in yeast)[56] (Figure 2). In some circumstances, activation of the IRE1 endonuclease domain mediates the cleavage and degradation of other cell mRNAs[57] and this feature complements other cellular mechanisms to control global protein translation[58].

Upon activation, the ATF6 transcription factor relocates to the Golgi where it is cleaved by S1P and S2P proteases[59], resulting in the release of an amino-terminal fragment that translocates to the nucleus where it promotes expression of chaperones, modifying enzymes and genes that code for transcription factors such as *DNA damage-inducible transcript 3* [(*DDIT3*), also known as CCAAT/enhancer binding protein homologous protein (CHOP)] and X-box binding protein 1 (XBP1), which play an important role in ER stress induced apoptosis and proteostasis, respectively[60-62]. Although this was never fully investigated, it is tempting to speculate that poxviruses may somehow interact with IRE1/ATF6-dependent stress pathways as these are such central components during the unfolded protein response.

It is known that XBP1 can be activated by TLR-2 and TL4-4 stimulation in an IRE1 dependent manner; also known is the fact that Vaccinia virus and other chor-

dopoxviruses are able to interfere with TLR signaling. Therefore, this seems to be a virus-driven indirect strategy to down-modulate XBP1 activation. Because XBP1 has been shown to be important for sustained production of cytokines by macrophages, it seems logical that poxvirus may interfere with XBP1 activation as a way to cope both with the host innate responses as well as with the ER stress.

Another component of the UPR, PERK (also known as eIF2αK3) shares homology to the IRE1 structure and activation pathways but lacks the RNase domain of IRE1[63]. Like the IRE1 activation, the release of BiP from PERK triggers dimerization of the later and its transphosphorylation (Figure 2). The activated PERK dimer is capable of recognizing and phosphorylating the alpha subunit of the translation initiation factor *eIF2α* at serine 51, reducing the translation of virus and cell mRNAs[64] (Figure 2). On the other hand, eIF2α phosphorylation upregulates the translation of ATF4, which induces expression of CHOP, GADD34, ATF3[65-67] and other genes involved in processes that are usurped and modulated during poxvirus replication, including amino acids transport[11], glutathione metabolism[68] and control of oxidative stress[69]. Not surprisingly, poxviruses encode proteins that mimic eIF2α and act as a pseudosubstrate for PERK, consequently suppressing phosphorylation of eIF2α and the shutoff of viral protein synthesis[70,71] (Figure 2).

HOST TRANSLATIONAL SHUTOFF

Most viruses, as obligate intracellular parasites, lack most genes related to the transcriptional and translational machinery, including those coding for enzymes, transcriptional factors, ribosomal subunits, translation factors and transfer RNAs (tRNA). Poxviruses encode their own transcriptional machinery but, to ensure viral mRNA translation during productive infections, they must effectively govern the host translation apparatus while avoiding stress responses like the eIF2α phosphorylation mediated translation shutoff.

In addition to PERK, which is involved in responses to the proteostasis imbalance in the ER, three other stress-activated eIF2α kinases are capable of inducing a broad range of responses designed to protect the cell. Protein kinase R (PKR), heme-regulated inhibitor (HRI) and general control nonderepressible 2 (GCN2) respond to dsRNA, oxidative stress and nutrient deprivation, respectively[72-74]. PKR (also known as eIF2αK2) is activated in response to stress signals usually resulting from viral infections and, together with other sensing and responding pathways that lead to eIF2α inactivation, is part of the so called integrated stress response (ISR). Poxviruses evolved non-redundant strategies to suppress activation of ISR and collectively inhibit the host translational shutoff response. The best characterized poxvirus' strategy to evade ISR is the expression of a pleiotropic viral protein, encoded by *E3L* orthologous genes, which is able to bind dsRNA and inhibit PKR activation. Nonetheless, other viral proteins also play critical roles in this process, including those encoded by *K1L*, *C7L* and *CP77L* orthologues[75-77]. Poxviruses lacking *E3L* orthologous genes induce the formation of host-protein dense antiviral granules (AVGs) that suppress translation of viral but not stress-induced host mRNAs and thus inhibit poxvirus replication[78].

ISR activation often promotes the formation of ribonucleoprotein aggregates called stress granules (SGs) at random sites throughout the cytoplasm. These SGs function as a protection zone for host RNAs where they can be stored when intracellular conditions are harmful[79]. SGs are distinct from AVGs in function and composition but share some components, like mRNA and RNA binding proteins [including Ras GTPase-activating protein-binding protein 1, Caprin-1, TIA1 and mRNA poly(A) binding protein, PABP] and other translation initiation components [including eIF3H and eIF4A/E/G (eIF4F complex) with the exception of 40S ribosomal subunits and eIF3B which only localize to SGs][80,81]; both granules, nevertheless, are dependent on translation repression. In productive poxviral infections, some of these granule components (as well as eIF4E and eIF4G) are sequestered to viral factories where they assemble and form eIF4F complexes that act together with PABP to promote activation of mRNAs harboring 7-methyl GTP caps and poly(A) tails[82]. Poxvirus mRNAs are capped on their 5' ends by the action of a viral methyl transferase enzyme complex[83-85] and are also polyadenylated by a complex mechanism involving repetitive transcription of thymidylates in the sequence 3'-ATTTA-5' often present at the sites of transcriptional initiation[86,87]. By sequestering molecules that activate capped and polyadenylated mRNAs to the viral factories, poxviruses are able to vigorously boost the translation of their own mRNAs.

HYPOXIC RESPONSE

Molecular oxygen (O_2) is an essential element to aerobic organisms that serves as a key substrate in cellular metabolism and bioenergetics. Hypoxic stress response is the process by which cells react and adapt to an insufficient O_2 availability (or hypoxia)[88]. During hypoxic conditions, cells activate a number of adaptive responses to match O_2 supply with metabolic, bioenergetic and redox demands. The hypoxia-inducible factor-1 (HIF-1) is the key regulator of the cell resilience in response to O_2 deprivation and it is regulated by prolyl hydroxylase domain-containing enzymes (PHDs)[89,90]. HIFs are obligate heterodimers, consisting of an O_2-destructible α-subunit and O_2-indestructible β subunit, and under physiologically normal O_2 levels (normoxia), PHDs mediate hydroxylation of proline residues in the HIFα subunit, triggering their recognition and labeling by E3 ubiquitin ligases, which leads in turn to their proteasomal degradation[91,92]. PHD activities are regulated by O_2 availability and by cellular metabolites such as tricarboxylic acid cycle

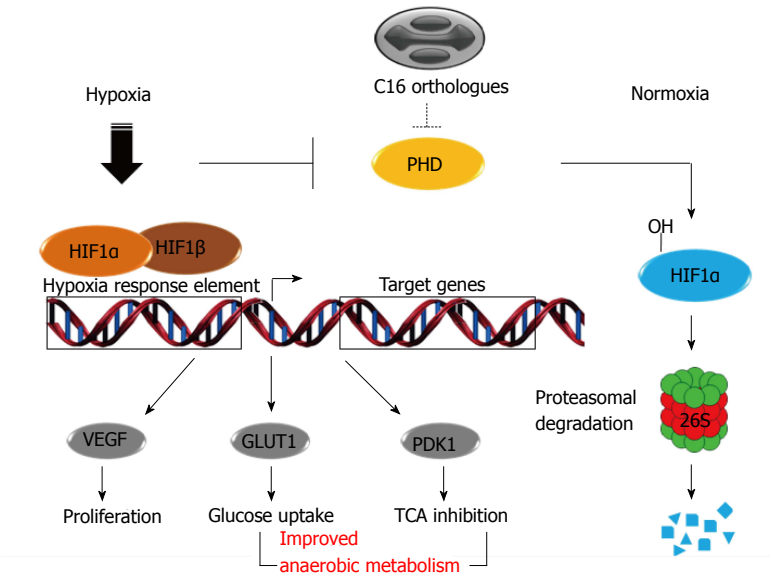

Figure 3 Hypoxic responses in poxviruses infected cells. Under normal O_2 disponibility (normoxia), HIF1α is hydroxylated on proline residues by PHDs. After that, HIF1α is recognized and ubiquitinated by E3 ubiquitin ligase and undergoes proteasomal degradation. Upon an insufficient O_2 availability (hypoxia), PHD become inactive and HIF1α forms heterodimers with HIF1β and triggers expression of regulators of TCA, cell proliferation and glucose metabolism. Poxviruses C16L orthologous genes code for proteins that inhibit PHD activities and result in expression of hypoxia target genes under normoxia conditions. HIF1: Hypoxia-inducible factor-1; PHD: Prolyl-hydroxylase domain-containing enzyme; TCA: Tricarboxylic acid cycle; VEGF: Endothelial growth factor; GLUT1: Glucose transporter-1; PDK1: Pyruvate dehydrogenase kinase-1.

(TCA) intermediates[93]. Due to the lack of sufficient O_2 upon hypoxia, PHDs become inactive and HIFα is consequently stabilized, causing the HIFs translocation to the nucleus where they bind to hypoxic responsive elements present in genes, such as *HSPA5*, *Fos*, *CXCR,* among other genes related to signal transduction, cell metabolism, apoptosis, *etc*[94-96] (Figure 3).

There are three PHD isoforms but PHD2 is believed to be the primary regulator of the HIF transcription factors[88]. The Vaccinia virus C16 protein is non-essential for virus replication but seems to play an important role in the down-modulation of the host immune responses[97]. Further studies showed that this protein can inhibit HIF1α hydroxylation through direct interaction with the PHD2 enzyme even when ectopically expressed[98]. Consequently, HIF1α is not ubiquitinated and degraded by proteasome, leading to the stabilization of this factor and up-regulation of HIF-responsive genes [endothelial growth factor (*VEGF*), glucose transporter-1 (*GLUT1*) and pyruvate dehydrogenase kinase-1 (*PDK1*)], improving cell metabolism and creating conditions that favor virus replication (Figure 3).

OXIDATIVE STRESS RESPONSE

Poxviruses exploit the *de novo* fatty acid biosynthesis in the cell and especially the production of palmitates. These molecules undergo β-oxidation in mitochondria and, together with the glutamine catabolism, generate acetyl-CoA and α-ketoglutarate, respectively. Both molecules enter in the TCA cycle and are used as major energy sources instead of glucose in infected cells[68,99,100]. In this metabolic pathway, O_2 plays a pivotal role as the final electron acceptor of oxidative phosphorylation coupled to the electron transfer chain, resulting in the production of water (H_2O), but also superoxide anion ($O_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), as well as other reactive oxygen species (ROS)[101,102] (Figure 4). ROS can significantly damage cell structures, causing lipoperoxidation, protein denaturation and DNA degradation; but on the other hand, ROS acts as a second messenger in mediating inflammation, stimulating cell proliferation and regulating apoptosis to maintain cell homeostasis[103]. Due to their cytotoxicity activity, cellular ROS levels are tightly limited by multiple detoxification processes such as antioxidant enzymes and vitamins whose functions are collectively appointed as an oxidative stress response[102].

ROS are usually controlled by antioxidant enzymes such as cooper/zinc-dependent superoxide dismutase (SOD) (cytoplasm), manganese-dependent SOD (mitochondria) and extracellular-SOD (also utilizes Cu/Zn as cofactor), which dismutate $O_2^{\bullet-}$ into H_2O_2. Hydrogen peroxides are in turn decomposed by catalase (CAT) and peroxidases such as glutathione peroxidase (GPx)[104] (Figure 4).

It has been shown that Myxoma virus and Shope fibroma virus increase intracellular ROS accumulation to promote growth of infected cells and immune evasion. This is achieved *via* inhibition of Cu/Zn-SOD1 activity through the expression of catalytically inactive homologs of cellular SOD1 that cannot bind Cu, which is essential for dismutase activity but retains the Zn-binding properties and, similarly to their cellular homologs, forms stable heterodimeric complexes with cellular Cu-dependent chaperones that are essential for SOD1 function[69,105] (Figure 4). It is likely that other poxviruses cause a similar effect during their multiplication cycle as some encode SOD-1 like genes; one such example is the A45R SOD-1-like gene from Vaccinia virus. Besides the SOD1 homologues, another known poxvirus gene product that can alter the redox state in infected cells is the Molluscum contagiosum virus MC066L gene product, which is homologous to the human GPx[12], an enzyme able to protect cells from the proapoptotic peroxides generated by ultraviolet (UV) light[106] (Figure 4).

Cellular peroxiredoxins and thioredoxins, among other host proteins that are not essential to the cellular redox state (*e.g.*, 60S ribosomal proteins, HGM1 and

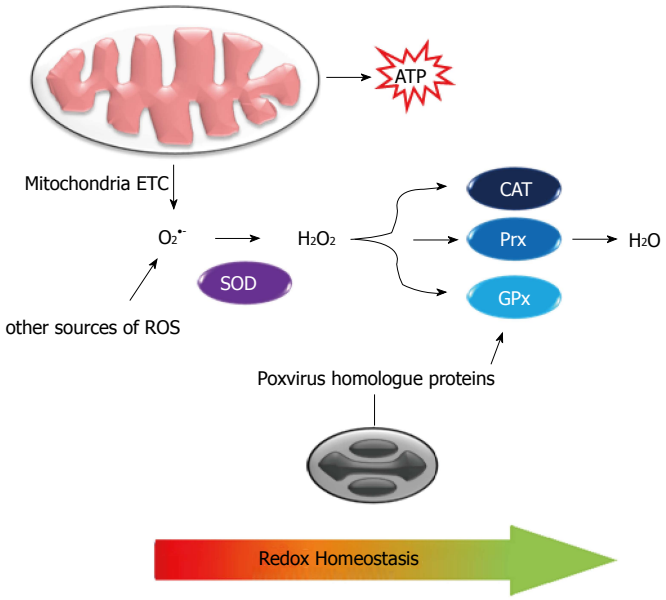

Figure 4 Role of poxvirus proteins in cell redox homeostasis. ROS are produced during physiological and stress conditions, for instance, during energetic metabolism in the mitochondria, and are detoxified by cellular enzymes (SOD, CAT, Prx, GPx) into water and oxygen. Poxviruses code proteins with homology to SOD, inhibiting the conversion of superoxide into hydrogen peroxide. Furthermore, MC066L gene product is homologous to the human GPx and can protect host cells of peroxide accumulation. ROS: Reactive oxygen species; SOD: Superoxide dismutase; CAT: Catalase; Prx: Peroxiredoxin; GPx: Glutathione peroxidase.

Rab10), can be detected in IMV particles. It has been speculated that those redox regulation proteins may play some role in virion maturation[38]. Indeed, redox conditions seem to be so important for poxviruses that many of them encode their own redox machinery in order to mediate disulfide bond formation in newly made viral proteins[107-109].

DNA DAMAGE RESPONSE

Several reports correlate stressful conditions with DNA damage responses (DDR). Hypoxia, ROS accumulation, ER stress, heat shock and mainly UV light exposure are conditions that either result or are resultant from DNA damage and whose sensing by the cell might contribute to the global stress adaptation response fostering cell resilience[96,110-114]. DDR events operate in diverse biological settings such as telomere homeostasis and generation of immune-receptor diversity[115] and include cell cycle checkpoint control, transcription, activation of DNA repair pathways, senescence and/or apoptosis. DNA damage can be subdivided into a few major types, including DNA double-strand breaks (DSB), DNA nucleotide adduct formation and base modification, DNA base pairing mismatches and single-strand breaks (SSB) which are caused by exposure to chemotherapeutic agents or environmental genotoxic agents such as polycyclic hydrocarbons and UV radiation. Accordingly, the major classes of DNA repair are DNA dsb repair by homologous recombination (HR) or nonhomologous end-joining (NHEJ), nucleotide excision repair, base-excision repair (BER), the Fanconi anemia/BRCA pathway and nucleotide mismatch repair[116]. The central sensor proteins in the DDR signal transduction cascade (ataxia telangiectasia mutated-ATM, ataxia telangiectasia and Rad3 related-ATR, DNA-dependent protein kinase-DNA-PKcs) belong to the phosphoinositide-3-kinase-related kinase (PIKK) family, with the exception of proteins from the PARP family which also respond to DNA lesions[117] (Figure 5).

ATM is recruited by the MRE-11-Rad50-NBS1 (MRN) complex to sites of DSBs and phosphorylates downstream substrates such as checkpoint kinase 2 (Chk2) which, subsequently phosphorylates p53 that in turn signals through p21 to slow the cycling of cells in order to facilitate DNA repair[118] (Figure 5). If the damage is too severe to be repaired, the cascade leads to death signalization through pro-apoptotic proteins. In the case of SSBs, ATR is recruited to damage sites in association with ATR-interacting protein by replication protein A (RPA). Once activated, these complexes phosphorylate Chk1 which, in turn, phosphorylates and inhibits cdc25c to mediate G_2/M arrest (or, alternatively, phosphorylates cdc25a to promote S-phase arrest). Most ATR substrates can also be phosphorylated by ATM and the major functions of ATR and ATM in cell cycle control are overlapping but non-redundant[119,120]. These signaling cascades appear to be the major repair pathways influenced by poxvirus infections (Figure 5) as they favor cell cycle progression to G_1, S and G_2 phases but arrest cells in the G_2 phase. Indeed, there is a preferential accumulation of poxvirus infected cells in G_2/M phases concurrent with a decrease in the number of cells in the G_0/G_1 ones[121,122].

The NHEJ repair pathway is initiated by association of Ku70/80 proteins to the DNA ends and the subsequent recruitment of the DNA-dependent protein kinase catalytic subunit (DNA-PKcs)[123,124]. These proteins localize both in the nucleus and the cytoplasm and are key factors in the immune response signaling, acting as viral dsDNA sensors leading to the induction of interferon regulatory factor 3 (IRF3) in a TANK-binding kinase 1-dependent manner[125]. Counteracting this immune signaling, the Vaccinia virus produces the C16 protein early in infection, which can bind to Ku70 blocking DNA-PK recruitment to DNA and the N2 protein, a virulence fac-

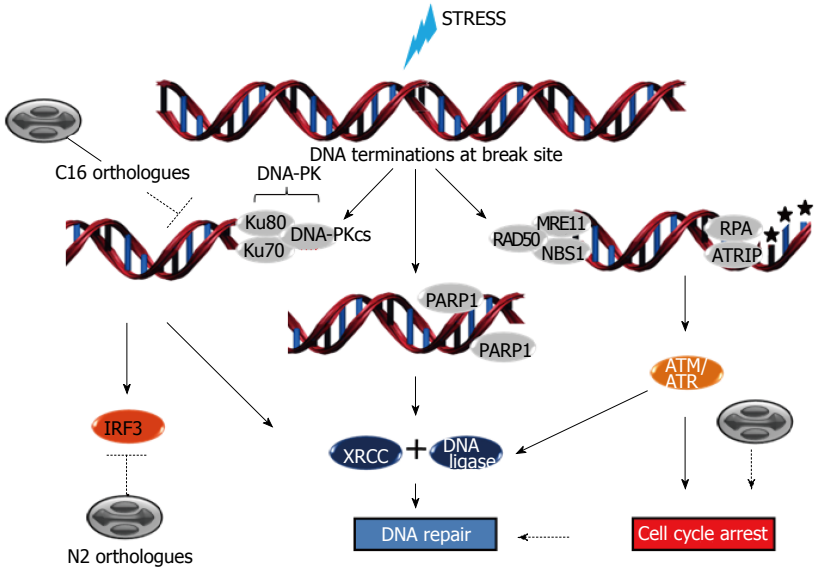

Figure 5 DNA damage responses and poxvirus infections. DNA breaks may be caused by many different sources. At sites of DNA double strand breaks (DSBs), DNA-PK is recruited by Ku proteins and induces DNA repair through XRCC4 and DNA ligase 4; DSBs also lead to the activation of the major interferon regulatory factor, IRF3. Upon DSBs occurrence, ataxia telangiectasia mutated (ATM) is recruited by the MRE-11-Rad50-NBS1 (MRN) complex to sites of broken DNA where they induce repair by XRCC2/3 and DNA ligase 1. ATM also controls cell cycle arrest which facilitates proper function of the DNA repair mechanisms. Upon single strand breaks, ataxia telangiectasia and Rad3 related (ATR) or poly (ADP-ribose) polymerase 1 (PARP1) are recruited to lesions sites and are activated, resulting in phosphorylation of downstream substrates, control of cell cycle arrest and/or repair of DNA lesions by XRCC1 and DNA ligase 3. Poxvirus infections affect cell cycle progression arresting cells in G_2 phase. They also encode C16 orthologues that bind to Ku70, blocking DNA-PK recruitment to broken DNA sites, and N2 orthologues, that inhibit IRF3-dependent innate immune responses. DNA-PK: DNA-dependent protein kinase; DNA-PKcs: DNA-dependent protein kinase catalytic subunit; RPA: Replication protein A.

tor that presents with the ability to inhibit IRF3-dependent innate immune responses[126,127] (Figure 5).

Poxviruses exploit their own replication machinery in order to repair eventual lesions at the viral DNA[3], mainly through the action of virally encoded uracil DNA glycosylases (coded by *D4R* orthologous genes), which initiate BER by hydrolyzing the glycosylic bond linking uracil to a deoxyribose sugar, and also through the repair of nicked duplex DNA substrate by a viral DNA ligase, a product of the *A50R* ORF present in some chordopoxviruses[128-131]. Furthermore, the viral DNA polymerase (coded by *E9L* gene orthologues) which possess 3' - 5' proofreading exonuclease activity and the *G5R* gene product which belongs to FEN1-like nucleases appear to conjunctly play important roles in viral DNA recombination through HR[132-136]. The cellular DNA ligase I can compensate an eventual absence of the viral DNA ligase and is recruited from the nucleus to the cytoplasmic viral factories. However, in the absence of a G5 protein, the viral DNA is fragmented and cannot be packaged[136,137].

MISCELLANEOUS CELL SIGNALING

PI3K/Akt signaling pathway

The phosphoinositide-3-kinase (PI3K) family of enzymes is grouped into three classes of proteins. PI3K is activated by G protein-coupled receptors and tyrosine kinase receptors to drive phosphorylation of inositol lipids at the 3' position of the inositol ring, generating lipid second messengers [3-phosphoinositides PI(3)P, PI(3,4)P_2 and PI(3,4,5)P_3][138,139]. Class IA PI3K proteins were shown to play an important role in poxvirus infections, promoting Akt phosphorylation and downstream events leading to the suppression of apoptosis, cell growth, survival and proliferation[140,141]. The PI3K/Akt pathway seems to be a determinant for the replicative success of Vaccinia virus and Cowpox virus, as well as for the host cell survival during infection, as the pharmacological impairment of the pathway components leads to diminished virus multiplication and apoptosis[141].

MAPK signaling pathway

Stress conditions (osmotic stress, ER stress, among others), growth factors and/or cytokines stimulate the activation of mitogen-activated protein kinases (MAPK)[142,143]. The MAPK family consists of a series of at least three main kinases active through distinct pathways: the extracellular signal-regulated protein kinases (ERKs), the c-Jun N-terminal kinases (JNKs) and the p38 family of kinases. These MAPK enzymes are activated by post-translational modifications induced by specific kinases, named MAPK kinases (MAP2K), which are activated by upstream MAPKK kinase (MAP3K) [Raf, MAPK/ERK kinase (MEKKs) and apoptosis signal-regulating kinase (ASK)][144] and which in turn respond either to external stimuli sensed by receptors on the cell surface or through interactions with GTP-binding proteins, among other kinases. Poxviruses have been shown to trigger mitogenic signals at early stages of infection, resulting in the expression of egr-1 and other genes, such as the proto-

oncogene c-fos, through the activation of ERK1/2. This process is essential for multiplication of some members of this viral family as blocking of those kinases hampers normal virus multiplication[145-147]. Additionally, the JNK pathway is also important for normal virus morphogenesis and accumulation of enveloped infectious forms[148] as blocking of the pathway influences cell-to-cell virus spread.

CONCLUSION

The activation of cellular stress responses in infected cells is a complex process that promotes simultaneously both cell resilience and death mechanisms upon a viral infection. In order to achieve replicative success in such conditions, poxviruses must subvert these cell responses to their own benefit. Members of the Poxviridae family are fully geared up to interfere with and manipulate cell fate in a way that very few other animal viruses do. They have unique abilities to turn off and/or combat negative effects of stress responses while still fomenting mechanisms to support the completion of its life cycle. Overall, poxviruses modulate the activation of a network of protein kinases (PI3K, PIKKs, MAPKs) and other enzymatic post-translational modifiers, such as the ubiquitin ligases and proteins involved in cell reprogramming (including ATFs, HSFs, XBP1, HIFs), while selectively inhibiting the activation or expression of host proteins (DNA-PK, IRF3, PHDs, PKR, PERK among others). In parallel, they are able affect the cell metabolism and redox state, maintaining proteostasis (through HSPs and other hosts and viral chaperones) and controlling cell cycle and proliferation in order to establish a proper cell environment for virus replication. Many of these strategies are highly conserved among different poxviruses, while a few others are species-specific[149]. The evidence of horizontal gene transfer from host to virus, coupled with the proposed model of poxvirus genome evolution based on a simple mechanism of recombination-driven genomic expansions and contractions (which facilitates the rapid evolution of virus populations with otherwise low mutation rates), sheds light on how these viruses acquired this impressive number of strategies to wisely control their replication niche[150-152].

Over 50 years after the discovery of HSR by Ferruccio Ritossa[153], the cellular stress response knowledge is still growing (including specific organelle stress such as mitochondrial or peroxisomal UPR, Golgi stress response and so on) and the understanding of mechanisms by which poxviruses and other complex viruses interfere with stress responses can further illuminate the web of pathways regulating cell homeostasis, as well as how viruses intertwine their own biochemical needs into this intricate scenario.

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REFERENCES

1 International Committee on Taxonomy of Viruses (ICTV). Virus Taxonomy: 2013 Release. 2014; Available from: URL: http: //www.ictvonline.org/virusTaxonomy.asp
2 **Smith GL**. Rapid spreading and immune evasion by vaccinia virus. *Adv Exp Med Biol* 2014; **808**: 65-76 [PMID: 24595611 DOI: 10.1007/978-81-322-1774-9_6]
3 **Moss B**. Poxvirus DNA replication. *Cold Spring Harb Perspect Biol* 2013; **5**: [PMID: 23838441 DOI: 10.1101/cshperspect.a010199]
4 **Katsafanas GC**, Moss B. Colocalization of transcription and translation within cytoplasmic poxvirus factories coordinates viral expression and subjugates host functions. *Cell Host Microbe* 2007; **2**: 221-228 [PMID: 18005740 DOI: 10.1016/j.chom.2007.08.005]
5 **Tolonen N**, Doglio L, Schleich S, Krijnse Locker J. Vaccinia virus DNA replication occurs in endoplasmic reticulum-enclosed cytoplasmic mini-nuclei. *Mol Biol Cell* 2001; **12**: 2031-2046 [PMID: 11452001]
6 **Schramm B**, Locker JK. Cytoplasmic organization of POXvirus DNA replication. *Traffic* 2005; **6**: 839-846 [PMID: 16138898 DOI: 10.1111/j.1600-0854.2005.00324.x]
7 **Smith GL**, Benfield CT, Maluquer de Motes C, Mazzon M, Ember SW, Ferguson BJ, Sumner RP. Vaccinia virus immune evasion: mechanisms, virulence and immunogenicity. *J Gen Virol* 2013; **94**: 2367-2392 [PMID: 23999164 DOI: 10.1099/vir.0.055921-0]
8 **Yang Z**, Bruno DP, Martens CA, Porcella SF, Moss B. Genome-wide analysis of the 5′ and 3′ ends of vaccinia virus early mRNAs delineates regulatory sequences of annotated and anomalous transcripts. *J Virol* 2011; **85**: 5897-5909 [PMID: 21490097 DOI: 10.1128/JVI.00428-11]
9 **Guerra S**, López-Fernández LA, Pascual-Montano A, Muñoz M, Harshman K, Esteban M. Cellular gene expression survey of vaccinia virus infection of human HeLa cells. *J Virol* 2003; **77**: 6493-6506 [PMID: 12743306]
10 **Jindal S**, Young RA. Vaccinia virus infection induces a stress response that leads to association of Hsp70 with viral proteins. *J Virol* 1992; **66**: 5357-5362 [PMID: 1501279]
11 **Pavon-Eternod M**, David A, Dittmar K, Berglund P, Pan T, Bennink JR, Yewdell JW. Vaccinia and influenza A viruses select rather than adjust tRNAs to optimize translation. *Nucleic Acids Res* 2013; **41**: 1914-1921 [PMID: 23254333 DOI: 10.1093/nar/gks986]
12 **Senkevich TG**, Bugert JJ, Sisler JR, Koonin EV, Darai G, Moss B. Genome sequence of a human tumorigenic poxvirus: prediction of specific host response-evasion genes. *Science* 1996; **273**: 813-816 [PMID: 8670425]
13 **Xiao A**, Wong J, Luo H. Viral interaction with molecular chaperones: role in regulating viral infection. *Arch Virol* 2010; **155**: 1021-1031 [PMID: 20461534 DOI: 10.1007/s00705-010-0691-3]
14 **Bisht H**, Brown E, Moss B. Kinetics and intracellular location of intramolecular disulfide bond formation mediated by the cytoplasmic redox system encoded by vaccinia virus. *Virology* 2010; **398**: 187-193 [PMID: 20042211 DOI: 10.1016/j.virol.2009.11.026]
15 **Myskiw C**, Arsenio J, Booy EP, Hammett C, Deschambault Y, Gibson SB, Cao J. RNA species generated in vaccinia virus infected cells activate cell type-specific MDA5 or RIG-I dependent interferon gene transcription and PKR dependent apoptosis. *Virology* 2011; **413**: 183-193 [PMID: 21354589 DOI: 10.1016/j.virol.2011.01.034]
16 **Willis KL**, Langland JO, Shisler JL. Viral double-stranded RNAs from vaccinia virus early or intermediate gene transcripts possess PKR activating function, resulting in NF-kappaB activation, when the K1 protein is absent or mutated. *J Biol Chem* 2011; **286**: 7765-7778 [PMID: 21183678 DOI:

10.1074/jbc.M110.194704]
17 **Ritossa F.** A new puffing pattern induced by temperature shock and DNP in drosophila. *Experientia* 1962; **18**: 571-573 [DOI: 10.1007/BF02172188]
18 **Tissières A**, Mitchell HK, Tracy UM. Protein synthesis in salivary glands of Drosophila melanogaster: relation to chromosome puffs. *J Mol Biol* 1974; **84**: 389-398 [PMID: 4219221]
19 **Mirault ME**, Goldschmidt-Clermont M, Moran L, Arrigo AP, Tissières A. The effect of heat shock on gene expression in Drosophila melanogaster. *Cold Spring Harb Symp Quant Biol* 1978; **42** Pt 2: 819-827 [PMID: 98271]
20 **Kampinga HH**, Craig EA. The HSP70 chaperone machinery: J proteins as drivers of functional specificity. *Nat Rev Mol Cell Biol* 2010; **11**: 579-592 [PMID: 20651708 DOI: 10.1038/nrm2941]
21 **Arya R**, Mallik M, Lakhotia SC. Heat shock genes - integrating cell survival and death. *J Biosci* 2007; **32**: 595-610 [PMID: 17536179]
22 **Pelham HR**. A regulatory upstream promoter element in the Drosophila hsp 70 heat-shock gene. *Cell* 1982; **30**: 517-528 [PMID: 6814763]
23 **Akerfelt M**, Morimoto RI, Sistonen L. Heat shock factors: integrators of cell stress, development and lifespan. *Nat Rev Mol Cell Biol* 2010; **11**: 545-555 [PMID: 20628411 DOI: 10.1038/nrm2938]
24 **Westerheide SD**, Anckar J, Stevens SM, Sistonen L, Morimoto RI. Stress-inducible regulation of heat shock factor 1 by the deacetylase SIRT1. *Science* 2009; **323**: 1063-1066 [PMID: 19229036 DOI: 10.1126/science.1165946]
25 **Hietakangas V**, Ahlskog JK, Jakobsson AM, Hellesuo M, Sahlberg NM, Holmberg CI, Mikhailov A, Palvimo JJ, Pirkkala L, Sistonen L. Phosphorylation of serine 303 is a prerequisite for the stress-inducible SUMO modification of heat shock factor 1. *Mol Cell Biol* 2003; **23**: 2953-2968 [PMID: 12665592]
26 **Anckar J**, Hietakangas V, Denessiouk K, Thiele DJ, Johnson MS, Sistonen L. Inhibition of DNA binding by differential sumoylation of heat shock factors. *Mol Cell Biol* 2006; **26**: 955-964 [PMID: 16428449 DOI: 10.1128/MCB.26.3.955-964.2006]
27 **Victor M**, Benecke BJ. Expression levels of heat shock factors are not functionally coupled to the rate of expression of heat shock genes. *Mol Biol Rep* 1998; **25**: 135-141 [PMID: 9700049]
28 **Filone CM**, Caballero IS, Dower K, Mendillo ML, Cowley GS, Santagata S, Rozelle DK, Yen J, Rubins KH, Hacohen N, Root DE, Hensley LE, Connor J. The master regulator of the cellular stress response (HSF1) is critical for orthopoxvirus infection. *PLoS Pathog* 2014; **10**: e1003904 [PMID: 24516381 DOI: 10.1371/journal.ppat.1003904]
29 **Kowalczyk A**, Guzik K, Slezak K, Dziedzic J, Rokita H. Heat shock protein and heat shock factor 1 expression and localization in vaccinia virus infected human monocyte derived macrophages. *J Inflamm* (Lond) 2005; **2**: 12 [PMID: 16246258 DOI: 10.1186/1476-9255-2-12]
30 **Mihara M**, Hashizume M, Yoshida H, Suzuki M, Shiina M. IL-6/IL-6 receptor system and its role in physiological and pathological conditions. *Clin Sci* (Lond) 2012; **122**: 143-159 [PMID: 22029668 DOI: 10.1042/CS20110340]
31 **Sedger L**, Ramshaw I, Condie A, Medveczky J, Braithwaite A, Ruby J. Vaccinia virus replication is independent of cellular HSP72 expression which is induced during virus infection. *Virology* 1996; **225**: 423-427 [PMID: 8918931 DOI: 10.1006/viro.1996.0619]
32 **Sedger L**, Ruby J. Heat shock response to vaccinia virus infection. *J Virol* 1994; **68**: 4685-4689 [PMID: 8207845]
33 **Kotoglou P**, Kalaitzakis A, Vezyraki P, Tzavaras T, Michalis LK, Dantzer F, Jung JU, Angelidis C. Hsp70 translocates to the nuclei and nucleoli, binds to XRCC1 and PARP-1, and protects HeLa cells from single-strand DNA breaks. *Cell Stress Chaperones* 2009; **14**: 391-406 [PMID: 19089598 DOI: 10.1007/s12192-008-0093-6]
34 **Dickey CA**, Koren J, Zhang YJ, Xu YF, Jinwal UK, Birnbaum MJ, Monks B, Sun M, Cheng JQ, Patterson C, Bailey RM, Dunmore J, Soresh S, Leon C, Morgan D, Petrucelli L. Akt and CHIP coregulate tau degradation through coordinated interactions. *Proc Natl Acad Sci USA* 2008; **105**: 3622-3627 [PMID: 18292230 DOI: 10.1073/pnas.0709180105]
35 **Jacobs AT**, Marnett LJ. Systems analysis of protein modification and cellular responses induced by electrophile stress. *Acc Chem Res* 2010; **43**: 673-683 [PMID: 20218676 DOI: 10.1021/ar900286y]
36 **Hung JJ**, Chung CS, Chang W. Molecular chaperone Hsp90 is important for vaccinia virus growth in cells. *J Virol* 2002; **76**: 1379-1390 [PMID: 11773412]
37 **Castro AP**, Carvalho TM, Moussatché N, Damaso CR. Redistribution of cyclophilin A to viral factories during vaccinia virus infection and its incorporation into mature particles. *J Virol* 2003; **77**: 9052-9068 [PMID: 12885921]
38 **Chung CS**, Chen CH, Ho MY, Huang CY, Liao CL, Chang W. Vaccinia virus proteome: identification of proteins in vaccinia virus intracellular mature virion particles. *J Virol* 2006; **80**: 2127-2140 [PMID: 16474121 DOI: 10.1128/JVI.80.5.2127-2140.2006]
39 **Fagone P**, Jackowski S. Membrane phospholipid synthesis and endoplasmic reticulum function. *J Lipid Res* 2009; **50** Suppl: S311-S316 [PMID: 18952570 DOI: 10.1194/jlr.R800049-JLR200]
40 **Taylor RC**, Berendzen KM, Dillin A. Systemic stress signalling: understanding the cell non-autonomous control of proteostasis. *Nat Rev Mol Cell Biol* 2014; **15**: 211-217 [PMID: 24556842 DOI: 10.1038/nrm3752]
41 **Walter P**, Ron D. The unfolded protein response: from stress pathway to homeostatic regulation. *Science* 2011; **334**: 1081-1086 [PMID: 22116877 DOI: 10.1126/science.1209038]
42 **Marciniak SJ**, Ron D. Endoplasmic reticulum stress signaling in disease. *Physiol Rev* 2006; **86**: 1133-1149 [PMID: 17015486 DOI: 10.1152/physrev.00015.2006]
43 **Hollien J**. Evolution of the unfolded protein response. *Biochim Biophys Acta* 2013; **1833**: 2458-2463 [PMID: 23369734 DOI: 10.1016/j.bbamcr.2013.01.016]
44 **Shen J**, Snapp EL, Lippincott-Schwartz J, Prywes R. Stable binding of ATF6 to BiP in the endoplasmic reticulum stress response. *Mol Cell Biol* 2005; **25**: 921-932 [PMID: 15657421 DOI: 10.1128/MCB.25.3.921-932.2005]
45 **Li J**, Ni M, Lee B, Barron E, Hinton DR, Lee AS. The unfolded protein response regulator GRP78/BiP is required for endoplasmic reticulum integrity and stress-induced autophagy in mammalian cells. *Cell Death Differ* 2008; **15**: 1460-1471 [PMID: 18551133 DOI: 10.1038/cdd.2008.81]
46 **Li G**, Scull C, Ozcan L, Tabas I. NADPH oxidase links endoplasmic reticulum stress, oxidative stress, and PKR activation to induce apoptosis. *J Cell Biol* 2010; **191**: 1113-1125 [PMID: 21135141 DOI: 10.3109/1040841X.2013.813899]
47 **Maruri-Avidal L**, Weisberg AS, Bisht H, Moss B. Analysis of viral membranes formed in cells infected by a vaccinia virus L2-deletion mutant suggests their origin from the endoplasmic reticulum. *J Virol* 2013; **87**: 1861-1871 [PMID: 23192873 DOI: 10.1128/JVI.02779-12]
48 **Maruri-Avidal L**, Weisberg AS, Moss B. Vaccinia virus L2 protein associates with the endoplasmic reticulum near the growing edge of crescent precursors of immature virions and stabilizes a subset of viral membrane proteins. *J Virol* 2011; **85**: 12431-12441 [PMID: 21917978 DOI: 10.1128/JVI.05573-11]
49 **Maruri-Avidal L**, Weisberg AS, Moss B. Association of the vaccinia virus A11 protein with the endoplasmic reticulum and crescent precursors of immature virions. *J Virol* 2013; **87**: 10195-10206 [PMID: 23864611 DOI: 10.1128/JVI.01601-13]
50 **Versteeg GA**, van de Nes PS, Bredenbeek PJ, Spaan WJ.

The coronavirus spike protein induces endoplasmic reticulum stress and upregulation of intracellular chemokine mRNA concentrations. *J Virol* 2007; **81**: 10981-10990 [PMID: 17670839 DOI: 10.1128/JVI.01033-07]

51 **Pincus D**, Chevalier MW, Aragón T, van Anken E, Vidal SE, El-Samad H, Walter P. BiP binding to the ER-stress sensor Ire1 tunes the homeostatic behavior of the unfolded protein response. *PLoS Biol* 2010; **8**: e1000415 [PMID: 20625545 DOI: 10.1371/journal.pbio.1000415]

52 **Lisbona F**, Rojas-Rivera D, Thielen P, Zamorano S, Todd D, Martinon F, Glavic A, Kress C, Lin JH, Walter P, Reed JC, Glimcher LH, Hetz C. BAX inhibitor-1 is a negative regulator of the ER stress sensor IRE1alpha. *Mol Cell* 2009; **33**: 679-691 [PMID: 19328063 DOI: 10.1016/j.molcel.2009.02.017]

53 **Korennykh AV**, Egea PF, Korostelev AA, Finer-Moore J, Stroud RM, Zhang C, Shokat KM, Walter P. Cofactor-mediated conformational control in the bifunctional kinase/RNase Ire1. *BMC Biol* 2011; **9**: 48 [PMID: 21729334 DOI: 10.1186/1741-7007-9-48]

54 **Korennykh AV**, Korostelev AA, Egea PF, Finer-Moore J, Stroud RM, Zhang C, Shokat KM, Walter P. Structural and functional basis for RNA cleavage by Ire1. *BMC Biol* 2011; **9**: 47 [PMID: 21729333 DOI: 10.1186/1741-7007-9-47]

55 **Hetz C**. The biological meaning of the UPR. *Nat Rev Mol Cell Biol* 2013; **14**: 404 [PMID: 23756618 DOI: 10.1038/nrm3606]

56 **Calfon M**, Zeng H, Urano F, Till JH, Hubbard SR, Harding HP, Clark SG, Ron D. IRE1 couples endoplasmic reticulum load to secretory capacity by processing the XBP-1 mRNA. *Nature* 2002; **415**: 92-96 [PMID: 11780124 DOI: 10.1038/415092a]

57 **Hollien J**, Lin JH, Li H, Stevens N, Walter P, Weissman JS. Regulated Ire1-dependent decay of messenger RNAs in mammalian cells. *J Cell Biol* 2009; **186**: 323-331 [PMID: 19651891 DOI: 10.1083/jcb.200903014]

58 **Scheuner D**, Kaufman RJ. The unfolded protein response: a pathway that links insulin demand with beta-cell failure and diabetes. *Endocr Rev* 2008; **29**: 317-333 [PMID: 18436705 DOI: 10.1210/er.2007-0039]

59 **Mori K**. Signalling pathways in the unfolded protein response: development from yeast to mammals. *J Biochem* 2009; **146**: 743-750 [PMID: 19861400 DOI: 10.1093/jb/mvp166]

60 **Nishitoh H**. CHOP is a multifunctional transcription factor in the ER stress response. *J Biochem* 2012; **151**: 217-219 [PMID: 22210905 DOI: 10.1093/jb/mvr143]

61 **Liou HC**, Boothby MR, Finn PW, Davidon R, Nabavi N, Zeleznik-Le NJ, Ting JP, Glimcher LH. A new member of the leucine zipper class of proteins that binds to the HLA DR alpha promoter. *Science* 1990; **247**: 1581-1584 [PMID: 2321018]

62 **Yoshida H**, Matsui T, Yamamoto A, Okada T, Mori K. XBP1 mRNA is induced by ATF6 and spliced by IRE1 in response to ER stress to produce a highly active transcription factor. *Cell* 2001; **107**: 881-891 [PMID: 11779464]

63 **Harding HP**, Zhang Y, Ron D. Protein translation and folding are coupled by an endoplasmic-reticulum-resident kinase. *Nature* 1999; **397**: 271-274 [PMID: 9930704 DOI: 10.1038/16729]

64 **Jackson RJ**, Hellen CU, Pestova TV. The mechanism of eukaryotic translation initiation and principles of its regulation. *Nat Rev Mol Cell Biol* 2010; **11**: 113-127 [PMID: 20094052 DOI: 10.1038/nrm2838]

65 **Hussain SG,** Ramaiah KV. Endoplasmic Reticulum: Stress, signalling and apoptosis. *Current Science* 2007; **91**: 1684-1696

66 **Hussain SG**, Ramaiah KV. Reduced eIF2alpha phosphorylation and increased proapoptotic proteins in aging. *Biochem Biophys Res Commun* 2007; **355**: 365-370 [PMID: 17300747 DOI: 10.1016/j.bbrc.2007.01.156]

67 **Lee do Y**, Lee KS, Lee HJ, Kim do H, Noh YH, Yu K, Jung HY, Lee SH, Lee JY, Youn YC, Jeong Y, Kim DK, Lee WB, Kim SS. Activation of PERK signaling attenuates Abeta-mediated ER stress. *PLoS One* 2010; **5**: e10489 [PMID: 20463975 DOI: 10.1371/journal.pone.0010489]

68 **Fontaine KA**, Camarda R, Lagunoff M. Vaccinia virus requires glutamine but not glucose for efficient replication. *J Virol* 2014; **88**: 4366-4374 [PMID: 24501408 DOI: 10.1128/JVI.03134-13]

69 **Teoh ML**, Turner PV, Evans DH. Tumorigenic poxviruses up-regulate intracellular superoxide to inhibit apoptosis and promote cell proliferation. *J Virol* 2005; **79**: 5799-5811 [PMID: 15827194 DOI: 10.1128/JVI.79.9.5799-5811.2005]

70 **Kawagishi-Kobayashi M**, Cao C, Lu J, Ozato K, Dever TE. Pseudosubstrate inhibition of protein kinase PKR by swine pox virus C8L gene product. *Virology* 2000; **276**: 424-434 [PMID: 11040133 DOI: 10.1006/viro.2000.0561]

71 **Davies MV**, Elroy-Stein O, Jagus R, Moss B, Kaufman RJ. The vaccinia virus K3L gene product potentiates translation by inhibiting double-stranded-RNA-activated protein kinase and phosphorylation of the alpha subunit of eukaryotic initiation factor 2. *J Virol* 1992; **66**: 1943-1950 [PMID: 1347793]

72 **Williams BR**. PKR; a sentinel kinase for cellular stress. *Oncogene* 1999; **18**: 6112-6120 [PMID: 10557102 DOI: 10.1038/sj.onc.1203127]

73 **Zhang P**, McGrath BC, Reinert J, Olsen DS, Lei L, Gill S, Wek SA, Vattem KM, Wek RC, Kimball SR, Jefferson LS, Cavener DR. The GCN2 eIF2alpha kinase is required for adaptation to amino acid deprivation in mice. *Mol Cell Biol* 2002; **22**: 6681-6688 [PMID: 12215525]

74 **Miksanova M**, Igarashi J, Minami M, Sagami I, Yamauchi S, Kurokawa H, Shimizu T. Characterization of heme-regulated eIF2alpha kinase: roles of the N-terminal domain in the oligomeric state, heme binding, catalysis, and inhibition. *Biochemistry* 2006; **45**: 9894-9905 [PMID: 16893190 DOI: 10.1021/bi060556k]

75 **Backes S**, Sperling KM, Zwilling J, Gasteiger G, Ludwig H, Kremmer E, Schwantes A, Staib C, Sutter G. Viral host-range factor C7 or K1 is essential for modified vaccinia virus Ankara late gene expression in human and murine cells, irrespective of their capacity to inhibit protein kinase R-mediated phosphorylation of eukaryotic translation initiation factor 2alpha. *J Gen Virol* 2010; **91**: 470-482 [PMID: 19846675 DOI: 10.1099/vir.0.015347-0]

76 **Meng X**, Schoggins J, Rose L, Cao J, Ploss A, Rice CM, Xiang Y. C7L family of poxvirus host range genes inhibits antiviral activities induced by type I interferons and interferon regulatory factor 1. *J Virol* 2012; **86**: 4538-4547 [PMID: 22345458 DOI: 10.1128/JVI.06140-11]

77 **Li G**, Scull C, Ozcan L, Tabas I. NADPH oxidase links endoplasmic reticulum stress, oxidative stress, and PKR activation to induce apoptosis. *J Cell Biol* 2010; **191**: 1113-1125 [PMID: 21135141 DOI: 10.1083/jcb.201006121]

78 **Rozelle DK**, Filone CM, Kedersha N, Connor JH. Activation of stress response pathways promotes formation of antiviral granules and restricts virus replication. *Mol Cell Biol* 2014; **34**: 2003-2016 [PMID: 24662051 DOI: 10.1128/MCB.01630-13]

79 **Nover L**, Scharf KD, Neumann D. Cytoplasmic heat shock granules are formed from precursor particles and are associated with a specific set of mRNAs. *Mol Cell Biol* 1989; **9**: 1298-1308 [PMID: 2725500]

80 **Simpson-Holley M**, Kedersha N, Dower K, Rubins KH, Anderson P, Hensley LE, Connor JH. Formation of antiviral cytoplasmic granules during orthopoxvirus infection. *J Virol* 2011; **85**: 1581-1593 [PMID: 21147913 DOI: 10.1128/JVI.02247-10]

81 **Anderson P**, Kedersha N. RNA granules. *J Cell Biol* 2006; **172**: 803-808 [PMID: 16520386 DOI: 10.1083/jcb.200512082]

82 **Walsh D**, Arias C, Perez C, Halladin D, Escandon M, Ueda T, Watanabe-Fukunaga R, Fukunaga R, Mohr I. Eukaryotic translation initiation factor 4F architectural alterations ac-

company translation initiation factor redistribution in poxvirus-infected cells. *Mol Cell Biol* 2008; **28**: 2648-2658 [PMID: 18250159 DOI: 10.1128/MCB.01631-07]
83 **Boone RF**, Moss B. Methylated 5′-terminal sequences of vaccinia virus mRNA species made in vivo at early and late times after infection. *Virology* 1977; **79**: 67-80 [PMID: 867823]
84 **Martin SA**, Moss B. Modification of RNA by mRNA guanylyltransferase and mRNA (guanine-7-)methyltransferase from vaccinia virions. *J Biol Chem* 1975; **250**: 9330-9335 [PMID: 1194287]
85 **Venkatesan S**, Gershowitz A, Moss B. Modification of the 5′ end of mRNA. Association of RNA triphosphatase with the RNA guanylyltransferase-RNA (guanine-7-)methyltransferase complex from vaccinia virus. *J Biol Chem* 1980; **255**: 903-908 [PMID: 6243301]
86 **Ink BS**, Pickup DJ. Vaccinia virus directs the synthesis of early mRNAs containing 5′ poly(A) sequences. *Proc Natl Acad Sci USA* 1990; **87**: 1536-1540 [PMID: 1968253]
87 **Ahn BY**, Moss B. Capped poly(A) leaders of variable lengths at the 5′ ends of vaccinia virus late mRNAs. *J Virol* 1989; **63**: 226-232 [PMID: 2462059]
88 **Bagnall J**, Leedale J, Taylor SE, Spiller DG, White MR, Sharkey KJ, Bearon RN, Sée V. Tight control of hypoxia-inducible factor-α transient dynamics is essential for cell survival in hypoxia. *J Biol Chem* 2014; **289**: 5549-5564 [PMID: 24394419 DOI: 10.1074/jbc.M113.500405]
89 **Karuppagounder SS**, Ratan RR. Hypoxia-inducible factor prolyl hydroxylase inhibition: robust new target or another big bust for stroke therapeutics? *J Cereb Blood Flow Metab* 2012; **32**: 1347-1361 [PMID: 22415525 DOI: 10.1038/jcbfm.2012.28]
90 **Rabinowitz MH**. Inhibition of hypoxia-inducible factor prolyl hydroxylase domain oxygen sensors: tricking the body into mounting orchestrated survival and repair responses. *J Med Chem* 2013; **56**: 9369-9402 [PMID: 23977883 DOI: 10.1021/jm400386j]
91 **Buckley DL**, Van Molle I, Gareiss PC, Tae HS, Michel J, Noblin DJ, Jorgensen WL, Ciulli A, Crews CM. Targeting the von Hippel-Lindau E3 ubiquitin ligase using small molecules to disrupt the VHL/HIF-1α interaction. *J Am Chem Soc* 2012; **134**: 4465-4468 [PMID: 22369643 DOI: 10.1021/ja209924v]
92 **Essers PB,** Klasson TD, Pereboom TC, Mans DA, Nicastro M, Boldt K, Giles RH, Macinnes AW. The von Hippel-Lindau tumor suppressor regulates programmed cell death 5-mediated degradation of Mdm2. *Oncogene* 2014 Jan 27; Epub ahead of print [PMID: 24469044 DOI: 10.1038/onc.2013.598]
93 **Kaelin WG**, Ratcliffe PJ. Oxygen sensing by metazoans: the central role of the HIF hydroxylase pathway. *Mol Cell* 2008; **30**: 393-402 [PMID: 18498744 DOI: 10.1016/j.molcel.2008.04.009]
94 **Simon MC**, Keith B. The role of oxygen availability in embryonic development and stem cell function. *Nat Rev Mol Cell Biol* 2008; **9**: 285-296 [PMID: 18285802 DOI: 10.1038/nrm2354]
95 **Wang WD**, Chen ZT, Li R, Li DZ, Duan YZ, Cao ZH. Enhanced efficacy of radiation-induced gene therapy in mice bearing lung adenocarcinoma xenografts using hypoxia responsive elements. *Cancer Sci* 2005; **96**: 918-924 [PMID: 16367913 DOI: 10.1111/j.1349-7006.2005.00129.x]
96 **Bouquet F**, Ousset M, Biard D, Fallone F, Dauvillier S, Frit P, Salles B, Muller C. A DNA-dependent stress response involving DNA-PK occurs in hypoxic cells and contributes to cellular adaptation to hypoxia. *J Cell Sci* 2011; **124**: 1943-1951 [PMID: 21576354 DOI: 10.1242/jcs.078030]
97 **Fahy AS**, Clark RH, Glyde EF, Smith GL. Vaccinia virus protein C16 acts intracellularly to modulate the host response and promote virulence. *J Gen Virol* 2008; **89**: 2377-2387 [PMID: 18796705 DOI: 10.1099/vir.0.2008/004895-0]
98 **Mazzon M**, Peters NE, Loenarz C, Krysztofinska EM, Ember SW, Ferguson BJ, Smith GL. A mechanism for induction of a hypoxic response by vaccinia virus. *Proc Natl Acad Sci USA* 2013; **110**: 12444-12449 [PMID: 23836663 DOI: 10.1073/pnas.1302140110]
99 **Greseth MD**, Traktman P. De novo fatty acid biosynthesis contributes significantly to establishment of a bioenergetically favorable environment for vaccinia virus infection. *PLoS Pathog* 2014; **10**: e1004021 [PMID: 24651651 DOI: 10.1371/journal.ppat.1004021]
100 **Chang CW**, Li HC, Hsu CF, Chang CY, Lo SY. Increased ATP generation in the host cell is required for efficient vaccinia virus production. *J Biomed Sci* 2009; **16**: 80 [PMID: 19725950 DOI: 10.1186/1423-0127-16-80]
101 **Murphy MP**. How mitochondria produce reactive oxygen species. *Biochem J* 2009; **417**: 1-13 [PMID: 19061483 DOI: 10.1042/BJ20081386]
102 **Scandalios JG**. Oxidative stress: molecular perception and transduction of signals triggering antioxidant gene defenses. *Braz J Med Biol Res* 2005; **38**: 995-1014 [PMID: 16007271]
103 **D'Autréaux B**, Toledano MB. ROS as signalling molecules: mechanisms that generate specificity in ROS homeostasis. *Nat Rev Mol Cell Biol* 2007; **8**: 813-824 [PMID: 17848967 DOI: 10.1038/nrm2256]
104 **Liu N**, Lin Z, Guan L, Gaughan G, Lin G. Antioxidant enzymes regulate reactive oxygen species during pod elongation in Pisum sativum and Brassica chinensis. *PLoS One* 2014; **9**: e87588 [PMID: 24503564 DOI: 10.1371/journal.pone.0087588]
105 **Teoh ML**, Walasek PJ, Evans DH. Leporipoxvirus Cu,Zn-superoxide dismutase (SOD) homologs are catalytically inert decoy proteins that bind copper chaperone for SOD. *J Biol Chem* 2003; **278**: 33175-33184 [PMID: 12773543 DOI: 10.1074/jbc.M300644200]
106 **Shisler JL**, Senkevich TG, Berry MJ, Moss B. Ultraviolet-induced cell death blocked by a selenoprotein from a human dermatotropic poxvirus. *Science* 1998; **279**: 102-105 [PMID: 9417017]
107 **Senkevich TG**, White CL, Koonin EV, Moss B. Complete pathway for protein disulfide bond formation encoded by poxviruses. *Proc Natl Acad Sci USA* 2002; **99**: 6667-6672 [PMID: 11983854 DOI: 10.1073/pnas.062163799]
108 **Senkevich TG**, White CL, Weisberg A, Granek JA, Wolffe EJ, Koonin EV, Moss B. Expression of the vaccinia virus A2.5L redox protein is required for virion morphogenesis. *Virology* 2002; **300**: 296-303 [PMID: 12350360]
109 **White CL**, Senkevich TG, Moss B. Vaccinia virus G4L glutaredoxin is an essential intermediate of a cytoplasmic disulfide bond pathway required for virion assembly. *J Virol* 2002; **76**: 467-472 [PMID: 11752136]
110 **Cadet J**, Wagner JR. DNA base damage by reactive oxygen species, oxidizing agents, and UV radiation. *Cold Spring Harb Perspect Biol* 2013; **5**: [PMID: 23378590 DOI: 10.1101/cshperspect.a012559]
111 **Jena NR**. DNA damage by reactive species: Mechanisms, mutation and repair. *J Biosci* 2012; **37**: 503-517 [PMID: 22750987]
112 **Yuzefovych LV**, LeDoux SP, Wilson GL, Rachek LI. Mitochondrial DNA damage via augmented oxidative stress regulates endoplasmic reticulum stress and autophagy: crosstalk, links and signaling. *PLoS One* 2013; **8**: e83349 [PMID: 24349491 DOI: 10.1371/journal.pone.0083349]
113 **Yuzefovych LV**, Musiyenko SI, Wilson GL, Rachek LI. Mitochondrial DNA damage and dysfunction, and oxidative stress are associated with endoplasmic reticulum stress, protein degradation and apoptosis in high fat diet-induced insulin resistance mice. *PLoS One* 2013; **8**: e54059 [PMID: 23342074 DOI: 10.1371/journal.pone.0054059]
114 **Banerjee G**, Gupta N, Kapoor A, Raman G. UV induced bystander signaling leading to apoptosis. *Cancer Lett* 2005; **223**: 275-284 [PMID: 15896462 DOI: 10.1016/j.canlet.2004.09.035]
115 **Jackson SP**, Bartek J. The DNA-damage response in human

biology and disease. *Nature* 2009; **461**: 1071-1078 [PMID: 19847258 DOI: 10.1038/nature08467]
116 **Ghosal G**, Chen J. DNA damage tolerance: a double-edged sword guarding the genome. *Transl Cancer Res* 2013; **2**: 107-129 [PMID: 24058901]
117 **Abraham RT**. PI 3-kinase related kinases: 'big' players in stress-induced signaling pathways. *DNA Repair* (Amst) 2004; **3**: 883-887 [PMID: 15279773 DOI: 10.1016/j.dnarep.2004.04.002]
118 **Lavin MF**. Ataxia-telangiectasia: from a rare disorder to a paradigm for cell signalling and cancer. *Nat Rev Mol Cell Biol* 2008; **9**: 759-769 [PMID: 18813293 DOI: 10.1038/nrm2514]
119 **Harper JW**, Elledge SJ. The DNA damage response: ten years after. *Mol Cell* 2007; **28**: 739-745 [PMID: 18082599 DOI: 10.1016/j.molcel.2007.11.015]
120 **Christmann M,** Kaina B. Transcriptional regulation of human DNA repair genes following genotoxic stress: trigger mechanisms, inducible responses and genotoxic adaptation. *Nucleic Acids Res* 2013; **41**: 8403-8420 [DOI: 10.1093/nar/gkt635]
121 **Alkhalil A**, Hammamieh R, Hardick J, Ichou MA, Jett M, Ibrahim S. Gene expression profiling of monkeypox virus-infected cells reveals novel interfaces for host-virus interactions. *Virol J* 2010; **7**: 173 [PMID: 20667104 DOI: 10.1186/1743-422X-7-173]
122 **Wali A**, Strayer DS. Infection with vaccinia virus alters regulation of cell cycle progression. *DNA Cell Biol* 1999; **18**: 837-843 [PMID: 10595397 DOI: 10.1089/104454999314836]
123 **Sancar A**, Lindsey-Boltz LA, Unsal-Kaçmaz K, Linn S. Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. *Annu Rev Biochem* 2004; **73**: 39-85 [PMID: 15189136 DOI: 10.1146/annurev.biochem.73.011303.073723]
124 **Davis AJ**, Chen DJ. DNA double strand break repair via non-homologous end-joining. *Transl Cancer Res* 2013; **2**: 130-143 [PMID: 24000320 DOI: 10.3978/j.issn.2218-676X.2013.04.02]
125 **Ferguson BJ**, Mansur DS, Peters NE, Ren H, Smith GL. DNA-PK is a DNA sensor for IRF-3-dependent innate immunity. *Elife* (Cambridge) 2012; **1**: e00047 [PMID: 23251783 DOI: 10.7554/eLife.00047]
126 **Peters NE**, Ferguson BJ, Mazzon M, Fahy AS, Krysztofinska E, Arribas-Bosacoma R, Pearl LH, Ren H, Smith GL. A mechanism for the inhibition of DNA-PK-mediated DNA sensing by a virus. *PLoS Pathog* 2013; **9**: e1003649 [PMID: 24098118 DOI: 10.1371/journal.ppat.1003649]
127 **Ferguson BJ**, Benfield CT, Ren H, Lee VH, Frazer GL, Strnadova P, Sumner RP, Smith GL. Vaccinia virus protein N2 is a nuclear IRF3 inhibitor that promotes virulence. *J Gen Virol* 2013; **94**: 2070-2081 [PMID: 23761407 DOI: 10.1099/vir.0.054114-0]
128 **Upton C**, Stuart DT, McFadden G. Identification of a poxvirus gene encoding a uracil DNA glycosylase. *Proc Natl Acad Sci USA* 1993; **90**: 4518-4522 [PMID: 8389453]
129 **Stuart DT**, Upton C, Higman MA, Niles EG, McFadden G. A poxvirus-encoded uracil DNA glycosylase is essential for virus viability. *J Virol* 1993; **67**: 2503-2512 [PMID: 8474156]
130 **Shuman S**. Vaccinia virus DNA ligase: specificity, fidelity, and inhibition. *Biochemistry* 1995; **34**: 16138-16147 [PMID: 8519771]
131 **Kerr SM**, Smith GL. Vaccinia virus encodes a polypeptide with DNA ligase activity. *Nucleic Acids Res* 1989; **17**: 9039-9050 [PMID: 2587253]
132 **Gammon DB**, Evans DH. The 3'-to-5' exonuclease activity of vaccinia virus DNA polymerase is essential and plays a role in promoting virus genetic recombination. *J Virol* 2009; **83**: 4236-4250 [PMID: 19224992 DOI: 10.1128/JVI.02255-08]
133 **Hamilton MD**, Evans DH. Enzymatic processing of replication and recombination intermediates by the vaccinia virus DNA polymerase. *Nucleic Acids Res* 2005; **33**: 2259-2268 [PMID: 15843688 DOI: 10.1093/nar/gki525]
134 **Hamilton MD**, Nuara AA, Gammon DB, Buller RM, Evans DH. Duplex strand joining reactions catalyzed by vaccinia virus DNA polymerase. *Nucleic Acids Res* 2007; **35**: 143-151 [PMID: 17158165 DOI: 10.1093/nar/gkl1015]
135 **Da Silva M**, Shen L, Tcherepanov V, Watson C, Upton C. Predicted function of the vaccinia virus G5R protein. *Bioinformatics* 2006; **22**: 2846-2850 [PMID: 17021162 DOI: 10.1093/bioinformatics/btl506]
136 **Senkevich TG**, Koonin EV, Moss B. Predicted poxvirus FEN1-like nuclease required for homologous recombination, double-strand break repair and full-size genome formation. *Proc Natl Acad Sci USA* 2009; **106**: 17921-17926 [PMID: 19805122 DOI: 10.1073/pnas.0909529106]
137 **Paran N**, De Silva FS, Senkevich TG, Moss B. Cellular DNA ligase I is recruited to cytoplasmic vaccinia virus factories and masks the role of the vaccinia ligase in viral DNA replication. *Cell Host Microbe* 2009; **6**: 563-569 [PMID: 20006844 DOI: 10.1016/j.chom.2009.11.005]
138 **Leevers SJ**, Vanhaesebroeck B, Waterfield MD. Signalling through phosphoinositide 3-kinases: the lipids take centre stage. *Curr Opin Cell Biol* 1999; **11**: 219-225 [PMID: 10209156]
139 **Akinleye A**, Avvaru P, Furqan M, Song Y, Liu D. Phosphatidylinositol 3-kinase (PI3K) inhibitors as cancer therapeutics. *J Hematol Oncol* 2013; **6**: 88 [PMID: 24261963 DOI: 10.1186/1756-8722-6-88]
140 **Wang G**, Barrett JW, Stanford M, Werden SJ, Johnston JB, Gao X, Sun M, Cheng JQ, McFadden G. Infection of human cancer cells with myxoma virus requires Akt activation via interaction with a viral ankyrin-repeat host range factor. *Proc Natl Acad Sci USA* 2006; **103**: 4640-4645 [PMID: 16537421 DOI: 10.1073/pnas.0509341103]
141 **Soares JA**, Leite FG, Andrade LG, Torres AA, De Sousa LP, Barcelos LS, Teixeira MM, Ferreira PC, Kroon EG, Souto-Padrón T, Bonjardim CA. Activation of the PI3K/Akt pathway early during vaccinia and cowpox virus infections is required for both host survival and viral replication. *J Virol* 2009; **83**: 6883-6899 [PMID: 19386722 DOI: 10.1128/JVI.00245-09]
142 **Winter-Vann AM**, Johnson GL. Integrated activation of MAP3Ks balances cell fate in response to stress. *J Cell Biochem* 2007; **102**: 848-858 [PMID: 17786929 DOI: 10.1002/jcb.21522]
143 **Yang SH**, Sharrocks AD, Whitmarsh AJ. MAP kinase signalling cascades and transcriptional regulation. *Gene* 2013; **513**: 1-13 [PMID: 23123731 DOI: 10.1016/j.gene.2012.10.033]
144 **Hirai S**, Izawa M, Osada S, Spyrou G, Ohno S. Activation of the JNK pathway by distantly related protein kinases, MEKK and MUK. *Oncogene* 1996; **12**: 641-650 [PMID: 8637721]
145 **de Magalhães JC**, Andrade AA, Silva PN, Sousa LP, Ropert C, Ferreira PC, Kroon EG, Gazzinelli RT, Bonjardim CA. A mitogenic signal triggered at an early stage of vaccinia virus infection: implication of MEK/ERK and protein kinase A in virus multiplication. *J Biol Chem* 2001; **276**: 38353-38360 [PMID: 11459835 DOI: 10.1074/jbc.M100183200]
146 **Andrade AA**, Silva PN, Pereira AC, De Sousa LP, Ferreira PC, Gazzinelli RT, Kroon EG, Ropert C, Bonjardim CA. The vaccinia virus-stimulated mitogen-activated protein kinase (MAPK) pathway is required for virus multiplication. *Biochem J* 2004; **381**: 437-446 [PMID: 15025565 DOI: 10.1042/BJ20031375]
147 **Silva PN**, Soares JA, Brasil BS, Nogueira SV, Andrade AA, de Magalhães JC, Bonjardim MB, Ferreira PC, Kroon EG, Bruna-Romero O, Bonjardim CA. Differential role played by the MEK/ERK/EGR-1 pathway in orthopoxviruses vaccinia and cowpox biology. *Biochem J* 2006; **398**: 83-95 [PMID: 16686604 DOI: 10.1042/BJ20060509]
148 **Pereira AC**, Leite FG, Brasil BS, Soares-Martins JA, Torres AA, Pimenta PF, Souto-Padrón T, Traktman P, Ferreira PC, Kroon EG, Bonjardim CA. A vaccinia virus-driven interplay between the MKK4/7-JNK1/2 pathway and cytoskeleton

reorganization. *J Virol* 2012; **86**: 172-184 [PMID: 22031940 DOI: 10.1128/JVI.05638-11]
149 **Bratke KA**, McLysaght A, Rothenburg S. A survey of host range genes in poxvirus genomes. *Infect Genet Evol* 2013; **14**: 406-425 [PMID: 23268114 DOI: 10.1016/j.meegid.2012.12.002]
150 **McLysaght A**, Baldi PF, Gaut BS. Extensive gene gain associated with adaptive evolution of poxviruses. *Proc Natl Acad Sci USA* 2003; **100**: 15655-15660 [PMID: 14660798 DOI: 10.1073/pnas.2136653100]
151 **Hughes AL**, Friedman R. Poxvirus genome evolution by gene gain and loss. *Mol Phylogenet Evol* 2005; **35**: 186-195 [PMID: 15737590 DOI: 10.1016/j.ympev.2004.12.008]
152 **Elde NC**, Child SJ, Eickbush MT, Kitzman JO, Rogers KS, Shendure J, Geballe AP, Malik HS. Poxviruses deploy genomic accordions to adapt rapidly against host antiviral defenses. *Cell* 2012; **150**: 831-841 [PMID: 22901812 DOI: 10.1016/j.cell.2012.05.049]
153 **De Maio A**, Santoro MG, Tanguay RM, Hightower LE. Ferruccio Ritossa's scientific legacy 50 years after his discovery of the heat shock response: a new view of biology, a new society, and a new journal. *Cell Stress Chaperones* 2012; **17**: 139-143 [PMID: 22252402 DOI: 10.1007/s12192-012-0320-z]

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REVIEW

DNA methylation in liver diseases

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Abstract

Recently, growing evidences show that the combination of epigenetic and genetic abnormalities contribute together to the development of liver diseases. DNA methylation is a very important epigenetic mechanism in human beings. It refers to addition of the methyl groups to DNA and mainly occurs at cytosine adjacent to guanine. DNA methylation is prevalent across human genome and is essential for the normal human development, while its dysfunction is associated with many human diseases. A deep understanding of DNA methylation may provide us deep insight into the origination of liver diseases. Also, it may provide us new tools for diseases diagnosis and prognosis prediction. This review summarized recent progress of DNA methylation study and provided an overview of DNA methylation and liver diseases. Meanwhile, the association between DNA methylation and liver diseases including hepatocellular carcinoma, liver fibrosis, nonalcoholic steatohepatitis and liver failure were extensively discussed. Finally, we discussed the potential of DNA methylation therapeutics for liver diseases and the value of DNA methylation as biomarkers for liver diseases diagnosis and prognosis prediction. This review aimed to provide the emerging DNA methylation information about liver diseases. It might provide essential information for managing and care of these patients.

Key words: DNA methylation; Liver diseases; Hepatocellular carcinoma; Liver fibrosis; Nonalcoholic steatohepatitis; Liver failure

Core tip: This review summarized recent progress of DNA methylation study and provided an overview of DNA methylation and liver diseases. The association between DNA methylation and liver diseases including hepatocellular carcinoma, liver fibrosis, nonalcoholic steatohepatitis or liver failure were extensively discussed. We also discussed the potential of DNA methylation as biomarkers and therapeutic targets for liver diseases. This review aimed to provide the emerging DNA methylation information about liver diseases. It might provide essential information for managing and care of these patients.

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INTRODUCTION

Because of the high prevalence, liver diseases have been studied systematically during the past few decades. Many studies focus on genetic defects[1] and genome-wide association studies do provide us great information about the pathogenesis of liver diseases[2]. However, many questions which cannot be totally illustrated by genetic mechanism still exist, which lead researchers to initiate

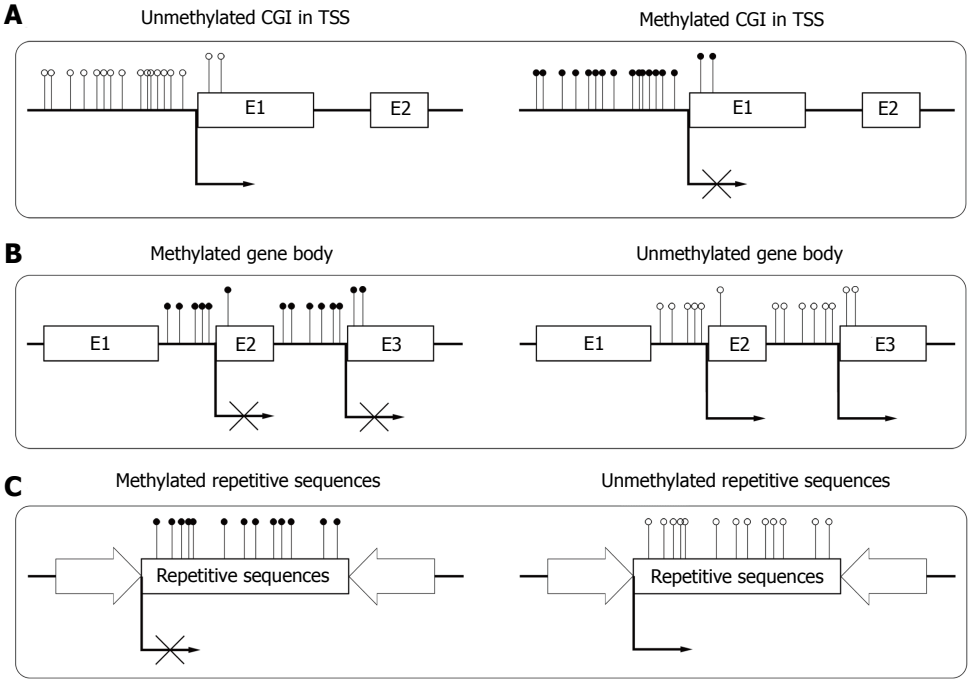

Figure 1 DNA methylation pattern in different parts of the genomes. The normal conditions are presented in the left column and aberrant conditions are shown on the right. The black dots represent methylated CpG sites and the white circles represent unmethylated CpG sites. A: In normal cells, CpG islands (CGI) in transcriptional start site (TSS) usually remain unmethylated, allowing transcription. Aberrant methylation often links to long-term stabilization of transcriptional silencing and loss of gene function both physically and pathologically; B: In normal cells, gene bodies are CpG-poor and extensively methylated, increasing elongation efficacy. Aberrant demethylation of gene bodies may facilitates spurious initiations of transcription; C: In normal cells, repetitive sequences of genome are highly methylated, preventing chromosomal instability or gene disruption. Aberrant demethylation of repetitive sequences may reactivate endoparasitic sequences.

the study of epigenetic variation. Recent studies showed that the combination of genetic and epigenetic variants contributed together to the susceptibility and progression of liver diseases[3-5]. Epigenetics refers to the heritable changes of gene expression without changes in gene sequence[6]. DNA methylation is a very important epigenetic mechanism in human and distribute widely across human genome. It is of crucial important for normal development, genomic imprinting as well as inactivation of X-chromosome[7-9]. Meanwhile, aberrant DNA methylation usually associates with many human diseases[10]. The goal of this article is to review the studies associated with DNA methylation and liver diseases. Finally, we look into the future prospect that DNA methylation may bring to the detection and treatment of liver diseases.

DNA METHYLATION AND ITS MECHANISM

DNA methylation which refers to addition of the methyl groups to DNA is firstly introduced in 1970s[11,12]. In invertebrates and fungi, DNA methylation only presents in small proportion of genome and varies among different clades[13,14]. In vertebrate genome, it presents in almost everywhere across the genome. Mainly, DNA methylation occurs at cytosine adjacent to guanine (CpG dinucleotides)[15]. In human genome, The CpG dinucleotides are very rare (approximately 1%). They are nonuniformly distributed and tend to cluster together to form CpG island (CGI). CGI refers to a 200-bp region in DNA which is characterized by high G+C content (more than 50%) and high observed CpG/expected CpG ratio (at least 0.6)[16]. Previous studies showed that CGIs existed in more than half of the genes in vertebrate genomes. Until now, the exact role of gene methylation in gene regulation remains largely unclear[17].

DNA methylation in transcriptional start sites

Until now, most of the studies on DNA methylation focus on CGIs in the transcriptional start sites (TSSs) of genes. In human genome, about 60% of gene TSSs contain CGIs and usually remain unmethylated in normal cells. Methylation of these CGIs often result in long-term stabilization of transcriptional silencing and loss of gene function both physically and pathologically[18] (Figure 1A). CpG island shore is defined as lower CpG density region which is close (approximately 2 kb) to the CGI. Recent studies show that most tissue specific methylation occurs at CpG island shores[19,20]. Aberrant DNA methylation at CpG island shores correlate even more strongly with gene expression than CGI[21].

There are about 40% of human genes which do not contain bona fide CGI at their TSSs[16]. Compared with genes that contained CGIs, the role of methylation in genes without CGIs at the TSSs has not been well demonstrated. More studies still need to be performed on genes without CGIs. Studies revealed that maspin gene had a promoter that did not reach the criteria for CGI

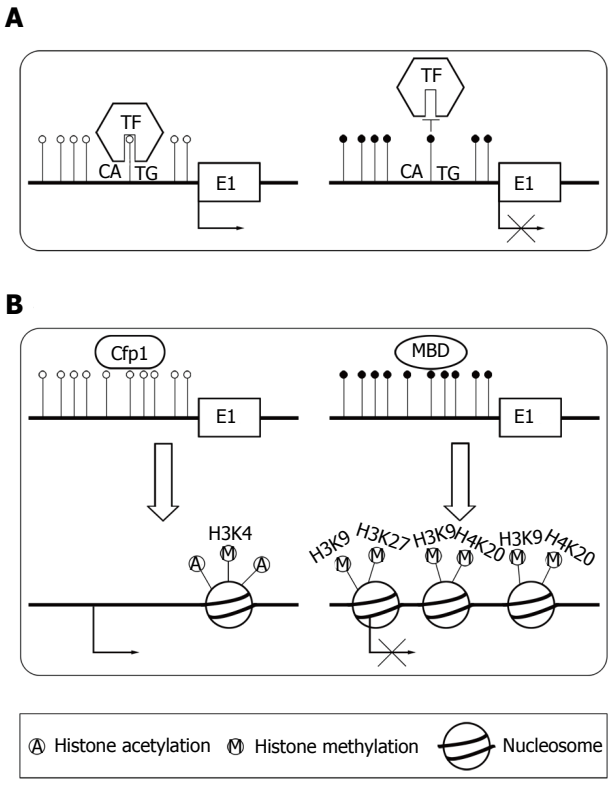

Figure 2 Transcriptional suppression mechanisms of DNA methylation in TSSs. The normal conditions are presented in the left column and aberrant conditions are shown on the right. The black dots represent methylated CpG sites and the white circles represent unmethylated CpG sites. A: In normal cells, transcription factors (TF) bind to unmethylated binding site, allowing transcription. Aberrant methylated binding site prevent TF binding to its normal sites; B: In normal cells, unmethylated CpG island can recruit CpG binding proteins (Cfp1) and trigger histone modifications characterized by high levels of acetylation and trimethylated H3K4, H3K36 and H3K79. Finally, it forms a structure suitable for transcription. Aberrant methylated recruit methyl-CpG-binding domain (MBD) proteins and trigger histone modifications characterized by high levels of H3K9, H3K27 and H4K20 methylation and low levels of acetylation. It represses the transcriptional permissiveness of chromatins and results in gene silencing.

and hypermethylation of this promoter was strongly correlated with its tissue specific expression[22]. However, *MAGE* gene was found to be unregulated by methylation in the promoters which do not satisfy CGIs.

There are two primary means by which DNA methylation in TSSs repress transcription. The transcription factors[23] control gene expression level. DNA methylation can directly preclude the transcription factors binding to its normal sites[24,25] (Figure 2A). For example, transcription factor YY1 which is essential for the imprinting of *Peg3* gene can bind to PEG3-DMR sequence in the first intron[24]. *In vivo*, the methylation of PEG3-DMR sequence precludes the binding of YY1, which result in the repression of maternal allele. In paternal allele, YY1 can effectively bind to the unmethylated PEG3-DMR sequence. Alternatively, DNA methylation can recruit specific proteins and induce a repressive chromatin structure[9] (Figure 2B). In normal condition, unmethylated CGIs can recruit CpG binding proteins, which form a structure suitable for transcription[26]. When CGIs are methylated, they can recruit methyl-CpG-binding domain (MBD) proteins[14,27]. Then, MBD proteins could recruit the histone modifying as well as chromatin remodeling complex to the methylated positions, which result in transcriptional silencing by repressing the transcriptional permissiveness of chromatins.

DNA methylation in gene bodies

Although CGIs also exist within gene bodies[28], most gene bodies are CpG-poor and extensively methylated. Studies showed that high level of gene body methylation was positively correlated with transcription, which meant it might associate with gene activation[29,30]. Zilberman *et al*[31] found that the methylation of gene body could increase elongation efficiency and prevent spurious initiations of transcription (Figure 1B). Shukla *et al*[32] illustrated that methylation between exons and introns was involved in regulating splicing[33]. Other studies reported that the methylation in gene body could be an important mechanism for managing promoter usage[34]. The high methylation level in gene body was essential for the elongation of a transcript.

DNA methylation in repetitive sequence

Repetitive elements comprise up to 45% of human genome[35], which mainly consist of interspersed repeats and tandem repeats. In normal somatic cells, repetitive sequences of genome are highly methylated. The deeply methylated condition is essential for the stability of chromosome and normal gene expression[36] (Figure 1C). Demethylation of repetitive sequences in genome may result in different kinds of diseases[37,38].

The inheritance of DNA methylation

DNA methylation is an important way to store hereditary information. Although it does not change gene sequence, it can propagate the methylation mark during cell divisions[39]. The DNA methylation inheritance process is catalyzed by DNA methyltransferase (DNMT) enzyme family. Manly, there are five members in DNMT enzyme family, DNMT1, DNMT2, DNMT3a, DNMT3b and DNMT3L. DNMT1, DNMT3a, DNMT3b serve as methyltransferase. Each of the three DNMTs is essential for normal human development[7,40]. Studies revealed that loss of methylation resulted from the inactivation of DNMTs could result in apoptosis of somatic cell[41] and cancer cells[42]. However, it showed that DNMTs were not essential for the survival of embryonic stem cells[43].

Bestor *et al*[44] firstly cloned DNMT1 in 1988 from mouse cells. Later studies revealed that DNMT1 expressed mostly at S phase of cell cycle[45] and mainly acted as maintenance DNMT. Interacting with the DNA polymerase processing factor proliferating cell nuclear antigen and ubiquitin-like plant homeodomain and RiNG finger domain containing protein 1 (UHRF1), DNMT1 methylated the hemimethylated sites during DNA semi-reserved replication[46,47]. Soon after replication, DNMT3a and DNMT3b bound to methylated DNA and corrected methylation sites missed by DNMT1 and completed the

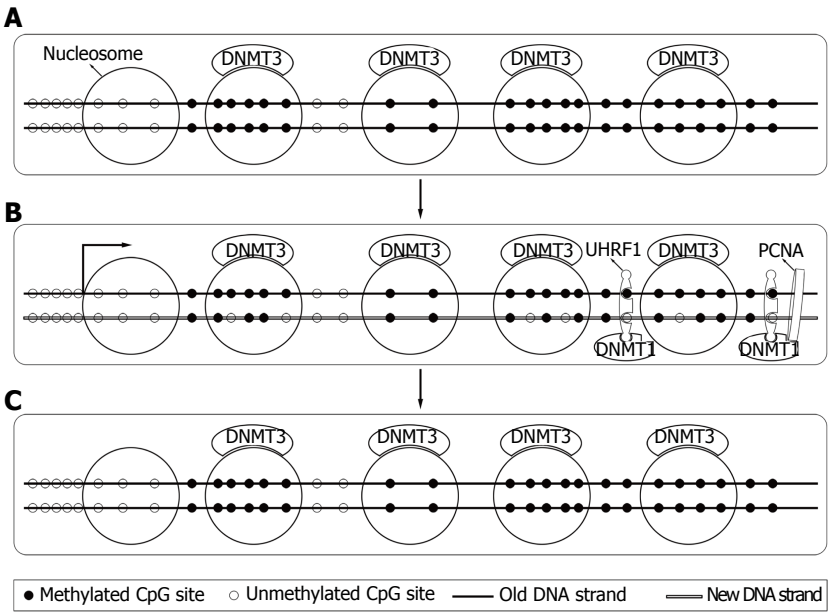

Figure 3 The maintenance of DNA methylation pattern. A: In somatic cells, DNA methyltransferase (DNMT) 3 (DNMT 3a and DNMT 3b) are bound to nucleosomes containing methylated DNA; B: During DNA semi-reserved replication, DNMT1 interact with the DNA polymerase processing factor proliferating cell nuclear antigen (PCNA) and ubiquitin-like plant homeodomain and RiNG finger domain containing protein 1 (UHRF1) and methylate the hemimethylated sites; C: Soon after DNA semi-reserved replication, DNMT3 correct methylation sites missed by DNMT1 and complete the process.

process[48,49] (Figure 3). DNMT1 was essential for both normal somatic cells and cancer cells and a knockout of DNMT1 could cause their death[41,42].

After the cloning of DNMT1, studies found that embryonic stem cells could still methylate retroviral DNA de novo even without DNMT1[50]. DNMT3a and DNMT3b were found in later studies[40]. They were regarded as de novo DNMT and functioned to set up normal methylation pattern during embryonic development. They were abundant in embryonic stem cell and less expressed in differentiated cells[51]. Other DNMTs like DNMT3L possessed no methylation catalytical activities. But Bourc'his *et al*[52] found that DNMT3L was crucial for establishment of maternal genomic imprinting.

DNA METHYLATION AND HEPATOCELLULAR CARCINOMA

In hepatocellular carcinoma (HCC), DNA methylation is characterized by a genome wide hypomethylation and a site specific hypermethylation[53]. Until now, many studies for presenting the DNA methylation patterns in HCC have been published.

Hypomethylation

Compared with normal liver tissue, DNA methylation in HCC is characterized by global hypomethylation. The hypomethylation of intergenic areas, repetitive DNA sequences[54], introns[55] and promoter CGI of specific oncogene[56] are responsible for the global hypomethylation. Global hypomethylation mainly result in chromosomal instability, loss of genomic imprinting[57,58] and reactivation of transposable elements, which may contribute to the development of cancer.

Previous studies revealed that the demethylation of chromosome 1 heterochromatin DNA was associated with the q-arm copy gain[59] in HCC. Also, a number of hypomethylated tumor-promoting genes, including HPA[60], MAT2A[61], VIM[62] and SNCG[63] have been identified in primary human HCC.

Hypermethylation

In tumor suppressor gene, the hypermethylation of CGIs in TSSs result in the loss of gene function, which is crucial for the origin of cancer[64]. The inactivation of tumor suppressor genes caused by hypermethylation of CGI in TSS exist in almost every type of human cancers[65]. Hypermethylation may affect the process of cell cycle regulation, DNA repair, angiogenesis, programmed cell death and tumor cell invasion. The genes silenced by hypermethylation in human cancers are often those who are essential for the maintenance of stem cell characteristics and/or the maturation of adult cells during cell renewal[65,66]. Silencing of these genes may result in the initiation of tumors through distribution of abnormal stem cells and/or abnormal of normal cell differentiation.

Until now, many tumor suppressor genes have been identified to be hypermethylated in HCC. Table 1 presents a group of frequently methylated genes in HCC.

DNA METHYLATION AND LIVER FIBROSIS

In liver fibrosis, aberrant DNA methylation has been studied for a few years. Until now, a number of aberrantly methylated genes have already been recognized. Through direct or indirect examination methods (treated with demethylating agents such as 5-aza-2'-deoxycytidine), these genes were identified to be aberrantly methylated. In activated hepatic stellate cell (HSC), transcriptional repression of some genes was indentified to be due to promoter hypermethylation of them.

Until now, genome-wide studies of DNA methylation associated with HSC activation were limited. Aberrant

Table 1 A selection of methylated genes in hepatocellular carcinoma

Gene	Location	Function	Methylation frequency	Ref.
GSTP1	11q13.2	Detoxification	41%-58%	[85-87]
SOCS1	16p13.13	Cytokine inhibitor	60%	[88]
RASSF1A	3p21.3	Apoptosis	54%-95%	[89,90]
E-Cadherin	16q22.1	Cell adhesion	33%-67%	[91,92]
APC	5q22.2	Signal transduction	46%	[93]
p16	9q21.3	CDK inhibitor	17%-83%	[94,95]
SFRP1	8p11.21	Signal transduction	59.50%	[96]
WIF-1	12q14.3	Signal transduction	61.90%	[97]
MGMT	10q26	DNA repair	22%-39%	[98,99]
TFPI2	7q21.3	Protease inhibitor	46.50%	[100]

methylation associated with HSC activation had been reported at specific loci such as the phosphatase and tensin homologue (*PTEN*) and patched1 (*PTCH1*) genes. These genes were aberrantly methylated in the myofibroblast and associated with the decreased of gene expression[67,68]. Our previous study revealed that aberrant promoter methylation of PPAR gamma gene was significantly associated with liver fibrosis in patients with chronic hepatitis B[69]. Other genes like Ras GTPase activating-like protein 1 (*RASAL1*) gene were also found to be aberrantly hypermethylated in liver fibrosis[70].

DNA METHYLATION AND NONALCOHOLIC STEATOHEPATITIS

So far, the relationship between DNA methylation and metabolic diseases was firmly established. Ahrens *et al*[71] used array-based DNA methylation and mRNA expression profiling to analyze the liver tissues from patients with non-alcoholic fatty liver disease (NAFLD) (n = 45) and health controls (n = 18). Aberrant methylation and decreased mRNA expression were seen for nine genes, which included genes for key enzymes in intermediate metabolism (ACLY, PC and PLCG1) and insulin or insulin-like signaling (IGFBP2, IGF1 and PRKCE)[71]. Studies showed that supplementation of diets lack of methyl donors could induce DNA hypomethylation and the development of steatosis in mice. However, supplementation of diets with methyl donors could prevent the development of NAFLD, suggesting that differences in the DNA methylation status might be a potential factor for individual susceptibilities to hepatic steatosis[72,73]. The supplementation of the maternal diet with methyl donors could induce aberrant methylation in adulthood and protect offspring from suffering obesity[74].

DNA METHYLATION AND LIVER FAILURE

Recent studies found that the aberrant methylation of several genes might participate in the development of liver failure. The aberrant promoter methylation of some anti-inflammatory genes might result in the down-regulate gene expression and inhibit their protective role in liver injury. Our previous study found that glutathione-S-transferase P1 (GSTP1) promoter hypermethylation occurred in patients with acute on chronic hepatitis B liver failure (ACHBLF) which might facilitate oxidative stress associated liver damage[75]. A study performed by Fan *et al*[76] showed that hypomethylation of IFN-γ gene promoter in peripheral blood mononuclear cells might be associated with the onset of ACHBLF. Qi *et al*[77] found that the aberrant hypermethylation of glutathione-S-transferase P1 (*GSTM3*) gene occurred in ACHBLF, which was correlated with their disease severity.

FURTHER PROSPECTS AND SUMMARY

The development of liver diseases is a multifactorial process characterized by the combination and integration of a multitude of alterations including genetic and epigenetic changes. In the past decades, there were exponential increases in the interest and progress of DNA methylation. Studies already revealed the potential role that DNA methylation played in the normal human development and initiation of diseases. DNA methylation-based biomarkers were proposed for disease risk assessment[78], early detection[79,80], prognostic prediction[81] and treatment outcome prediction of liver diseases[82]. Meanwhile, there was hope for developing therapeutic agents to manipulate aberrant DNA methylation patterns and to treat malignancies[6]. In 1970s, Constantinides *et al*[83] reported 5-azacytidine had remarkable effects on differentiated states of cells. In 2005, Brueckner *et al*[84] reported the drug RG101 could also reactivate tumor suppressor gene by inhibiting human DNA methyltransferase. Therefore, combined genetic and epigenetic information may help clinicians to prevent liver diseases developing in at-risk individuals and from passing on unhealthy DNA methylation characteristics to offsprings.

REFERENCES

1 **Mann DA.** Epigenetics in liver disease. *Hepatology* 2014; **60**: 1418-1425 [PMID: 24633972 DOI: 10.1002/hep.27131]
2 **Hindorff LA**, Sethupathy P, Junkins HA, Ramos EM, Mehta JP, Collins FS, Manolio TA. Potential etiologic and functional implications of genome-wide association loci for human diseases and traits. *Proc Natl Acad Sci USA* 2009; **106**: 9362-9367 [PMID: 19474294 DOI: 10.1073/pnas.0903103106]

3 **Timmer MR**, Beuers U, Fockens P, Ponsioen CY, Rauws EA, Wang KK, Krishnadath KK. Genetic and epigenetic abnormalities in primary sclerosing cholangitis-associated cholangiocarcinoma. *Inflamm Bowel Dis* 2013; **19**: 1789-1797 [PMID: 23615529 DOI: 10.1097/MIB.0b013e318281f49a]
4 **Ozen C**, Yildiz G, Dagcan AT, Cevik D, Ors A, Keles U, Topel H, Ozturk M. Genetics and epigenetics of liver cancer. *N Biotechnol* 2013; **30**: 381-384 [PMID: 23392071 DOI: 10.1016/j.nbt.2013.01.007]
5 **Zimmer V**, Lammert F. Genetics and epigenetics in the fibrogenic evolution of chronic liver diseases. *Best Pract Res Clin Gastroenterol* 2011; **25**: 269-280 [PMID: 21497744 DOI: 10.1016/j.bpg.2011.02.007]
6 **American Association for Cancer Research Human Epigenome Task Force,** European Union, Network of Excellence, Scientific Advisory Board. Moving AHEAD with an international human epigenome project. *Nature* 2008; **454**: 711-715 [PMID: 18685699 DOI: 10.1038/454711a]
7 **Li E**, Bestor TH, Jaenisch R. Targeted mutation of the DNA methyltransferase gene results in embryonic lethality. *Cell* 1992; **69**: 915-926 [PMID: 1606615]
8 **Reik W**, Walter J. Genomic imprinting: parental influence on the genome. *Nat Rev Genet* 2001; **2**: 21-32 [PMID: 11253064 DOI: 10.1038/35047554]
9 **Jaenisch R**, Bird A. Epigenetic regulation of gene expression: how the genome integrates intrinsic and environmental signals. *Nat Genet* 2003; **33** Suppl: 245-254 [PMID: 12610534 DOI: 10.1038/ng1089]
10 **Ray K**. NAFLD: Profiling NAFLD--liver gene expression and DNA methylation patterns to characterize disease severity. *Nat Rev Gastroenterol Hepatol* 2013; **10**: 565 [PMID: 23958603 DOI: 10.1038/nrgastro.2013.161]
11 **Riggs AD**. X inactivation, differentiation, and DNA methylation. *Cytogenet Cell Genet* 1975; **14**: 9-25 [PMID: 1093816]
12 **Holliday R**, Pugh JE. DNA modification mechanisms and gene activity during development. *Science* 1975; **187**: 226-232 [PMID: 1111098]
13 **Jones PA**. Functions of DNA methylation: islands, start sites, gene bodies and beyond. *Nat Rev Genet* 2012; **13**: 484-492 [PMID: 22641018 DOI: 10.1038/nrg3230]
14 **Deaton AM**, Bird A. CpG islands and the regulation of transcription. *Genes Dev* 2011; **25**: 1010-1022 [PMID: 21576262 DOI: 10.1101/gad.2037511]
15 **Lister R**, Pelizzola M, Dowen RH, Hawkins RD, Hon G, Tonti-Filippini J, Nery JR, Lee L, Ye Z, Ngo QM, Edsall L, Antosiewicz-Bourget J, Stewart R, Ruotti V, Millar AH, Thomson JA, Ren B, Ecker JR. Human DNA methylomes at base resolution show widespread epigenomic differences. *Nature* 2009; **462**: 315-322 [PMID: 19829295 DOI: 10.1038/nature08514]
16 **Takai D**, Jones PA. Comprehensive analysis of CpG islands in human chromosomes 21 and 22. *Proc Natl Acad Sci USA* 2002; **99**: 3740-3745 [PMID: 11891299 DOI: 10.1073/pnas.052410099]
17 **Schübeler D**. Molecular biology. Epigenetic islands in a genetic ocean. *Science* 2012; **338**: 756-757 [PMID: 23139324 DOI: 10.1126/science.1227243]
18 **Borgel J**, Guibert S, Li Y, Chiba H, Schübeler D, Sasaki H, Forné T, Weber M. Targets and dynamics of promoter DNA methylation during early mouse development. *Nat Genet* 2010; **42**: 1093-1100 [PMID: 21057502 DOI: 10.1038/ng.708]
19 **Irizarry RA**, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, Cui H, Gabo K, Rongione M, Webster M, Ji H, Potash JB, Sabunciyan S, Feinberg AP. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat Genet* 2009; **41**: 178-186 [PMID: 19151715 DOI: 10.1038/ng.298]
20 **Doi A**, Park IH, Wen B, Murakami P, Aryee MJ, Irizarry R, Herb B, Ladd-Acosta C, Rho J, Loewer S, Miller J, Schlaeger T, Daley GQ, Feinberg AP. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet* 2009; **41**: 1350-1353 [PMID: 19881528 DOI: 10.1038/ng.471]
21 **Ji H**, Ehrlich LI, Seita J, Murakami P, Doi A, Lindau P, Lee H, Aryee MJ, Irizarry RA, Kim K, Rossi DJ, Inlay MA, Serwold T, Karsunky H, Ho L, Daley GQ, Weissman IL, Feinberg AP. Comprehensive methylome map of lineage commitment from haematopoietic progenitors. *Nature* 2010; **467**: 338-342 [PMID: 20720541 DOI: 10.1038/nature09367]
22 **Futscher BW**, Oshiro MM, Wozniak RJ, Holtan N, Hanigan CL, Duan H, Domann FE. Role for DNA methylation in the control of cell type specific maspin expression. *Nat Genet* 2002; **31**: 175-179 [PMID: 12021783 DOI: 10.1038/ng886]
23 **Gal-Yam EN**, Jeong S, Tanay A, Egger G, Lee AS, Jones PA. Constitutive nucleosome depletion and ordered factor assembly at the GRP78 promoter revealed by single molecule footprinting. *PLoS Genet* 2006; **2**: e160 [PMID: 17002502]
24 **Kim J**, Kollhoff A, Bergmann A, Stubbs L. Methylation-sensitive binding of transcription factor YY1 to an insulator sequence within the paternally expressed imprinted gene, Peg3. *Hum Mol Genet* 2003; **12**: 233-245 [PMID: 12554678]
25 **Perini G**, Diolaiti D, Porro A, Della Valle G. In vivo transcriptional regulation of N-Myc target genes is controlled by E-box methylation. *Proc Natl Acad Sci USA* 2005; **102**: 12117-12122 [PMID: 16093321]
26 **Thomson JP**, Skene PJ, Selfridge J, Clouaire T, Guy J, Webb S, Kerr AR, Deaton A, Andrews R, James KD, Turner DJ, Illingworth R, Bird A. CpG islands influence chromatin structure via the CpG-binding protein Cfp1. *Nature* 2010; **464**: 1082-1086 [PMID: 20393567 DOI: 10.1038/nature08924]
27 **Lopez-Serra L**, Esteller M. Proteins that bind methylated DNA and human cancer: reading the wrong words. *Br J Cancer* 2008; **98**: 1881-1885 [PMID: 18542062 DOI: 10.1038/sj.bjc.6604374]
28 **Jones PA**. The DNA methylation paradox. *Trends Genet* 1999; **15**: 34-37 [PMID: 10087932]
29 **Wolf SF**, Jolly DJ, Lunnen KD, Friedmann T, Migeon BR. Methylation of the hypoxanthine phosphoribosyltransferase locus on the human X chromosome: implications for X-chromosome inactivation. *Proc Natl Acad Sci USA* 1984; **81**: 2806-2810 [PMID: 6585829]
30 **Hellman A**, Chess A. Gene body-specific methylation on the active X chromosome. *Science* 2007; **315**: 1141-1143 [PMID: 17322062]
31 **Zilberman D**, Gehring M, Tran RK, Ballinger T, Henikoff S. Genome-wide analysis of Arabidopsis thaliana DNA methylation uncovers an interdependence between methylation and transcription. *Nat Genet* 2007; **39**: 61-69 [PMID: 17128275]
32 **Shukla S**, Kavak E, Gregory M, Imashimizu M, Shutinoski B, Kashlev M, Oberdoerffer P, Sandberg R, Oberdoerffer S. CTCF-promoted RNA polymerase II pausing links DNA methylation to splicing. *Nature* 2011; **479**: 74-79 [PMID: 21964334 DOI: 10.1038/nature10442]
33 **Laurent L**, Wong E, Li G, Huynh T, Tsirigos A, Ong CT, Low HM, Kin Sung KW, Rigoutsos I, Loring J, Wei CL. Dynamic changes in the human methylome during differentiation. *Genome Res* 2010; **20**: 320-331 [PMID: 20133333 DOI: 10.1101/gr.101907.109]
34 **Maunakea AK**, Nagarajan RP, Bilenky M, Ballinger TJ, D'Souza C, Fouse SD, Johnson BE, Hong C, Nielsen C, Zhao Y, Turecki G, Delaney A, Varhol R, Thiessen N, Shchors K, Heine VM, Rowitch DH, Xing X, Fiore C, Schillebeeckx M, Jones SJ, Haussler D, Marra MA, Hirst M, Wang T, Costello JF. Conserved role of intragenic DNA methylation in regulating alternative promoters. *Nature* 2010; **466**: 253-257 [PMID: 20613842 DOI: 10.1038/nature09165]
35 **Lander ES,** Linton LM, Birren B, Nusbaum C, Zody MC, Baldwin J, Devon K, Dewar K, Doyle M, FitzHugh W,

Funke R, Gage D, Harris K, Heaford A, Howland J, Kann L, Lehoczky J, LeVine R, McEwan P, McKernan K, Meldrim J, Mesirov JP, Miranda C, Morris W, Naylor J, Raymond C, Rosetti M, Santos R, Sheridan A, Sougnez C, Stange-Thomann N, Stojanovic N, Subramanian A, Wyman D, Rogers J, Sulston J, Ainscough R, Beck S, Bentley D, Burton J, Clee C, Carter N, Coulson A, Deadman R, Deloukas P, Dunham A, Dunham I, Durbin R, French L, Grafham D, Gregory S, Hubbard T, Humphray S, Hunt A, Jones M, Lloyd C, McMurray A, Matthews L, Mercer S, Milne S, Mullikin JC, Mungall A, Plumb R, Ross M, Shownkeen R, Sims S, Waterston RH, Wilson RK, Hillier LW, McPherson JD, Marra MA, Mardis ER, Fulton LA, Chinwalla AT, Pepin KH, Gish WR, Chissoe SL, Wendl MC, Delehaunty KD, Miner TL, Delehaunty A, Kramer JB, Cook LL, Fulton RS, Johnson DL, Minx PJ, Clifton SW, Hawkins T, Branscomb E, Predki P, Richardson P, Wenning S, Slezak T, Doggett N, Cheng JF, Olsen A, Lucas S, Elkin C, Uberbacher E, Frazier M, Gibbs RA, Muzny DM, Scherer SE, Bouck JB, Sodergren EJ, Worley KC, Rives CM, Gorrell JH, Metzker ML, Naylor SL, Kucherlapati RS, Nelson DL, Weinstock GM, Sakaki Y, Fujiyama A, Hattori M, Yada T, Toyoda A, Itoh T, Kawagoe C, Watanabe H, Totoki Y, Taylor T, Weissenbach J, Heilig R, Saurin W, Artiguenave F, Brottier P, Bruls T, Pelletier E, Robert C, Wincker P, Smith DR, Doucette-Stamm L, Rubenfield M, Weinstock K, Lee HM, Dubois J, Rosenthal A, Platzer M, Nyakatura G, Taudien S, Rump A, Yang H, Yu J, Wang J, Huang G, Gu J, Hood L, Rowen L, Madan A, Qin S, Davis RW, Federspiel NA, Abola AP, Proctor MJ, Myers RM, Schmutz J, Dickson M, Grimwood J, Cox DR, Olson MV, Kaul R, Shimizu N, Kawasaki K, Minoshima S, Evans GA, Athanasiou M, Schultz R, Roe BA, Chen F, Pan H, Ramser J, Lehrach H, Reinhardt R, McCombie WR, de la Bastide M, Dedhia N, Blocker H, Hornischer K, Nordsiek G, Agarwala R, Aravind L, Bailey JA, Bateman A, Batzoglou S, Birney E, Bork P, Brown DG, Burge CB, Cerutti L, Chen HC, Church D, Clamp M, Copley RR, Doerks T, Eddy SR, Eichler EE, Furey TS, Galagan J, Gilbert JG, Harmon C, Hayashizaki Y, Haussler D, Hermjakob H, Hokamp K, Jang W, Johnson LS, Jones TA, Kasif S, Kaspryzk A, Kennedy S, Kent WJ, Kitts P, Koonin EV, Korf I, Kulp D, Lancet D, Lowe TM, McLysaght A, Mikkelsen T, Moran JV, Mulder N, Pollara VJ, Ponting CP, Schuler G, Schultz J, Slater G, Smit AF, Stupka E, Szustakowski J, Thierry-Mieg D, Thierry-Mieg J, Wagner L, Wallis J, Wheeler R, Williams A, Wolf YI, Wolfe KH, Yang SP, Yeh RF, Collins F, Guyer MS, Peterson J, Felsenfeld A, Wetterstrand KA, Patrinos A, Morgan MJ, de Jong P, Catanese JJ, Osoegawa K, Shizuya H, Choi S, Chen YJ. Initial sequencing and analysis of the human genome. *Nature* 2001; **409**: 860-921 [PMID: 11237011 DOI: 10.1038/35057062]

36 **Esteller M**. Cancer epigenomics: DNA methylomes and histone-modification maps. *Nat Rev Genet* 2007; **8**: 286-298 [PMID: 17339880]

37 **Moarefi AH**, Chédin F. ICF syndrome mutations cause a broad spectrum of biochemical defects in DNMT3B-mediated de novo DNA methylation. *J Mol Biol* 2011; **409**: 758-772 [PMID: 21549127 DOI: 10.1016/j.jmb.2011.04.050]

38 **Weisenberger DJ**, Campan M, Long TI, Kim M, Woods C, Fiala E, Ehrlich M, Laird PW. Analysis of repetitive element DNA methylation by MethyLight. *Nucleic Acids Res* 2005; **33**: 6823-6836 [PMID: 16326863]

39 **Song J**, Teplova M, Ishibe-Murakami S, Patel DJ. Structure-based mechanistic insights into DNMT1-mediated maintenance DNA methylation. *Science* 2012; **335**: 709-712 [PMID: 22323818 DOI: 10.1126/science.1214453]

40 **Okano M**, Bell DW, Haber DA, Li E. DNA methyltransferases Dnmt3a and Dnmt3b are essential for de novo methylation and mammalian development. *Cell* 1999; **99**: 247-257 [PMID: 10555141]

41 **Jackson-Grusby L**, Beard C, Possemato R, Tudor M, Fambrough D, Csankovszki G, Dausman J, Lee P, Wilson C, Lander E, Jaenisch R. Loss of genomic methylation causes p53-dependent apoptosis and epigenetic deregulation. *Nat Genet* 2001; **27**: 31-39 [PMID: 11137995 DOI: 10.1038/83730]

42 **Chen T**, Hevi S, Gay F, Tsujimoto N, He T, Zhang B, Ueda Y, Li E. Complete inactivation of DNMT1 leads to mitotic catastrophe in human cancer cells. *Nat Genet* 2007; **39**: 391-396 [PMID: 17322882]

43 **Woodward JJ**, Blair R. Redox modulation of N-methyl-D-aspartate-stimulated neurotransmitter release from rat brain slices. *J Neurochem* 1991; **57**: 2059-2064 [PMID: 1682419]

44 **Bestor T**, Laudano A, Mattaliano R, Ingram V. Cloning and sequencing of a cDNA encoding DNA methyltransferase of mouse cells. The carboxyl-terminal domain of the mammalian enzymes is related to bacterial restriction methyltransferases. *J Mol Biol* 1988; **203**: 971-983 [PMID: 3210246]

45 **Robertson KD**, Keyomarsi K, Gonzales FA, Velicescu M, Jones PA. Differential mRNA expression of the human DNA methyltransferases (DNMTs) 1, 3a and 3b during the G(0)/G(1) to S phase transition in normal and tumor cells. *Nucleic Acids Res* 2000; **28**: 2108-2113 [PMID: 10773079]

46 **Chuang LS**, Ian HI, Koh TW, Ng HH, Xu G, Li BF. Human DNA-(cytosine-5) methyltransferase-PCNA complex as a target for p21WAF1. *Science* 1997; **277**: 1996-2000 [PMID: 9302295]

47 **Bostick M**, Kim JK, Estève PO, Clark A, Pradhan S, Jacobsen SE. UHRF1 plays a role in maintaining DNA methylation in mammalian cells. *Science* 2007; **317**: 1760-1764 [PMID: 17673620]

48 **Jones PA**, Liang G. Rethinking how DNA methylation patterns are maintained. *Nat Rev Genet* 2009; **10**: 805-811 [PMID: 19789556 DOI: 10.1038/nrg2651]

49 **Sharma S**, De Carvalho DD, Jeong S, Jones PA, Liang G. Nucleosomes containing methylated DNA stabilize DNA methyltransferases 3A/3B and ensure faithful epigenetic inheritance. *PLoS Genet* 2011; **7**: e1001286 [PMID: 21304883 DOI: 10.1371/journal.pgen.1001286]

50 **Lei H**, Oh SP, Okano M, Jüttermann R, Goss KA, Jaenisch R, Li E. De novo DNA cytosine methyltransferase activities in mouse embryonic stem cells. *Development* 1996; **122**: 3195-3205 [PMID: 8898232]

51 **Esteller M**. Epigenetic gene silencing in cancer: the DNA hypermethylome. *Hum Mol Genet* 2007; **16** Spec No 1: R50-R59 [PMID: 17613547]

52 **Bourc'his D**, Xu GL, Lin CS, Bollman B, Bestor TH. Dnmt3L and the establishment of maternal genomic imprints. *Science* 2001; **294**: 2536-2539 [PMID: 11719692 DOI: 10.1126/science.1065848]

53 **Jones PA**, Baylin SB. The fundamental role of epigenetic events in cancer. *Nat Rev Genet* 2002; **3**: 415-428 [PMID: 12042769 DOI: 10.1038/nrg816]

54 **Goelz SE**, Vogelstein B, Hamilton SR, Feinberg AP. Hypomethylation of DNA from benign and malignant human colon neoplasms. *Science* 1985; **228**: 187-190 [PMID: 2579435]

55 **Feinberg AP**, Tycko B. The history of cancer epigenetics. *Nat Rev Cancer* 2004; **4**: 143-153 [PMID: 14732866 DOI: 10.1038/nrc1279]

56 **Feinberg AP**, Vogelstein B. Hypomethylation distinguishes genes of some human cancers from their normal counterparts. *Nature* 1983; **301**: 89-92 [PMID: 6185846]

57 **Eden A**, Gaudet F, Waghmare A, Jaenisch R. Chromosomal instability and tumors promoted by DNA hypomethylation. *Science* 2003; **300**: 455 [PMID: 12702868 DOI: 10.1126/science.1083557]

58 **Rodriguez J**, Frigola J, Vendrell E, Risques RA, Fraga MF, Morales C, Moreno V, Esteller M, Capellà G, Ribas M, Peinado MA. Chromosomal instability correlates with genome-wide DNA demethylation in human primary colorectal cancers. *Cancer Res* 2006; **66**: 8462-9468 [PMID: 16951157]

59 **Wong N**, Lam WC, Lai PB, Pang E, Lau WY, Johnson PJ.

Hypomethylation of chromosome 1 heterochromatin DNA correlates with q-arm copy gain in human hepatocellular carcinoma. *Am J Pathol* 2001; **159**: 465-471 [PMID: 11485905]
60 **Xiao Y**, Kleeff J, Shi X, Büchler MW, Friess H. Heparanase expression in hepatocellular carcinoma and the cirrhotic liver. *Hepatol Res* 2003; **26**: 192-198 [PMID: 12850691]
61 **Yang H**, Huang ZZ, Zeng Z, Chen C, Selby RR, Lu SC. Role of promoter methylation in increased methionine adenosyltransferase 2A expression in human liver cancer. *Am J Physiol Gastrointest Liver Physiol* 2001; **280**: G184-G190 [PMID: 11208539]
62 **Kitamura Y**, Shirahata A, Sakuraba K, Goto T, Mizukami H, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y, Hibi K. Aberrant methylation of the Vimentin gene in hepatocellular carcinoma. *Anticancer Res* 2011; **31**: 1289-1291 [PMID: 21508377]
63 **Zhao W**, Liu H, Liu W, Wu Y, Chen W, Jiang B, Zhou Y, Xue R, Luo C, Wang L, Jiang JD, Liu J. Abnormal activation of the synuclein-gamma gene in hepatocellular carcinomas by epigenetic alteration. *Int J Oncol* 2006; **28**: 1081-1088 [PMID: 16596223]
64 **Esteller M**. Epigenetics in cancer. *N Engl J Med* 2008; **358**: 1148-1159 [PMID: 18337604 DOI: 10.1056/NEJMra072067]
65 **Baylin SB**, Ohm JE. Epigenetic gene silencing in cancer - a mechanism for early oncogenic pathway addiction? *Nat Rev Cancer* 2006; **6**: 107-116 [PMID: 16491070]
66 **Kane MF**, Loda M, Gaida GM, Lipman J, Mishra R, Goldman H, Jessup JM, Kolodner R. Methylation of the hMLH1 promoter correlates with lack of expression of hMLH1 in sporadic colon tumors and mismatch repair-defective human tumor cell lines. *Cancer Res* 1997; **57**: 808-811 [PMID: 9041175]
67 **Bian EB**, Huang C, Ma TT, Tao H, Zhang H, Cheng C, Lv XW, Li J. DNMT1-mediated PTEN hypermethylation confers hepatic stellate cell activation and liver fibrogenesis in rats. *Toxicol Appl Pharmacol* 2012; **264**: 13-22 [PMID: 22841775 DOI: 10.1016/j.taap.2012.06.022]
68 **Yang JJ**, Tao H, Huang C, Shi KH, Ma TT, Bian EB, Zhang L, Liu LP, Hu W, Lv XW, Li J. DNA methylation and MeCP2 regulation of PTCH1 expression during rats hepatic fibrosis. *Cell Signal* 2013; **25**: 1202-1211 [PMID: 23333245 DOI: 10.1016/j.cellsig.2013.01.005]
69 **Zhao Q**, Fan YC, Zhao J, Gao S, Zhao ZH, Wang K. DNA methylation patterns of peroxisome proliferator-activated receptor gamma gene associated with liver fibrosis and inflammation in chronic hepatitis B. *J Viral Hepat* 2013; **20**: 430-437 [PMID: 23647960 DOI: 10.1111/jvh.12048]
70 **Tao H**, Huang C, Yang JJ, Ma TT, Bian EB, Zhang L, Lv XW, Jin Y, Li J. MeCP2 controls the expression of RASAL1 in the hepatic fibrosis in rats. *Toxicology* 2011; **290**: 327-333 [PMID: 22056649 DOI: 10.1016/j.tox.2011.10.011]
71 **Ahrens M**, Ammerpohl O, von Schönfels W, Kolarova J, Bens S, Itzel T, Teufel A, Herrmann A, Brosch M, Hinrichsen H, Erhart W, Egberts J, Sipos B, Schreiber S, Häsler R, Stickel F, Becker T, Krawczak M, Röcken C, Siebert R, Schafmayer C, Hampe J. DNA methylation analysis in nonalcoholic fatty liver disease suggests distinct disease-specific and remodeling signatures after bariatric surgery. *Cell Metab* 2013; **18**: 296-302 [PMID: 23931760 DOI: 10.1016/j.cmet.2013.07.004]
72 **Pogribny IP**, Tryndyak VP, Bagnyukova TV, Melnyk S, Montgomery B, Ross SA, Latendresse JR, Rusyn I, Beland FA. Hepatic epigenetic phenotype predetermines individual susceptibility to hepatic steatosis in mice fed a lipogenic methyl-deficient diet. *J Hepatol* 2009; **51**: 176-186 [PMID: 19450891 DOI: 10.1016/j.jhep.2009.03.021]
73 **Cordero P**, Campion J, Milagro FI, Martínez JA. Dietary supplementation with methyl donor groups could prevent nonalcoholic fatty liver. *Hepatology* 2011; **53**: 2151-2152 [PMID: 21538430 DOI: 10.1002/hep.24164]
74 **Dolinoy DC**, Weidman JR, Waterland RA, Jirtle RL. Maternal genistein alters coat color and protects Avy mouse offspring from obesity by modifying the fetal epigenome. *Environ Health Perspect* 2006; **114**: 567-572 [PMID: 16581547 DOI: 10.1289/ehp.8700]
75 **Li T**, Meng QH, Zou ZQ, Fan YC, Long B, Guo YM, Hou W, Zhao J, Li J, Yu HW, Zhu YK, Wang K. Correlation between promoter methylation of glutathione-S-tranferase P1 and oxidative stress in acute-on-chronic hepatitis B liver failure. *J Viral Hepat* 2011; **18**: e226-e231 [PMID: 21692937 DOI: 10.1111/j.1365-2893.2011.01438.x]
76 **Fan XP**, Zou ZQ, Long B, Guo YM, Wang SK, Jia DX, Xu AL, Li FC, Fan YC, Wang K. Enhanced demethylation of interferon-γ gene promoter in peripheral blood mononuclear cells is associated with acute-on-chronic hepatitis B liver failure. *Tohoku J Exp Med* 2011; **224**: 13-19 [PMID: 21505270]
77 **Qi L**, Zou ZQ, Wang LY, Gao S, Fan YC, Long B, Guo YM, Xu AL, Han J, Li T, Wang K. Methylation of the glutathione-S-transferase M3 gene promoter is associated with oxidative stress in acute-on-chronic hepatitis B liver failure. *Tohoku J Exp Med* 2012; **228**: 43-51 [PMID: 22976281]
78 **Mah WC**, Lee CG. DNA methylation: potential biomarker in Hepatocellular Carcinoma. *Biomark Res* 2014; **2**: 5 [PMID: 24635883 DOI: 10.1186/2050-7771-2-5]
79 **Ji XF**, Fan YC, Gao S, Yang Y, Zhang JJ, Wang K. MT1M and MT1G promoter methylation as biomarkers for hepatocellular carcinoma. *World J Gastroenterol* 2014; **20**: 4723-4729 [PMID: 24782625 DOI: 10.3748/wjg.v20.i16.4723]
80 **Han LY**, Fan YC, Mu NN, Gao S, Li F, Ji XF, Dou CY, Wang K. Aberrant DNA methylation of G-protein-coupled bile acid receptor Gpbar1 (TGR5) is a potential biomarker for hepatitis B Virus associated hepatocellular carcinoma. *Int J Med Sci* 2014; **11**: 164-171 [PMID: 24465162 DOI: 10.7150/ijms.6745]
81 **Honda S,** Haruta M, Sugawara W, Sasaki F, Ohira M, Matsunaga T, Yamaoka H, Horie H, Ohnuma N, Nakagawara A, Hiyama E, Todo S, Kaneko Y. Quantitative analysis of APC promoter methylation in hepatocellular carcinoma and its prognostic implications. *Oncol Lett* 2014; **7**: 1683-1688 [PMID: 24765201 DOI: 10.3892/ol.2014.1951]
82 **Honda S**, Haruta M, Sugawara W, Sasaki F, Ohira M, Matsunaga T, Yamaoka H, Horie H, Ohnuma N, Nakagawara A, Hiyama E, Todo S, Kaneko Y. The methylation status of RASSF1A promoter predicts responsiveness to chemotherapy and eventual cure in hepatoblastoma patients. *Int J Cancer* 2008; **123**: 1117-1125 [PMID: 18537155 DOI: 10.1002/ijc.23613]
83 **Constantinides PG**, Jones PA, Gevers W. Functional striated muscle cells from non-myoblast precursors following 5-azacytidine treatment. *Nature* 1977; **267**: 364-366 [PMID: 68440]
84 **Brueckner B**, Garcia Boy R, Siedlecki P, Musch T, Kliem HC, Zielenkiewicz P, Suhai S, Wiessler M, Lyko F. Epigenetic reactivation of tumor suppressor genes by a novel small-molecule inhibitor of human DNA methyltransferases. *Cancer Res* 2005; **65**: 6305-6311 [PMID: 16024632]
85 **Zhang YJ**, Chen Y, Ahsan H, Lunn RM, Chen SY, Lee PH, Chen CJ, Santella RM. Silencing of glutathione S-transferase P1 by promoter hypermethylation and its relationship to environmental chemical carcinogens in hepatocellular carcinoma. *Cancer Lett* 2005; **221**: 135-143 [PMID: 15808399]
86 **Zhong S**, Tang MW, Yeo W, Liu C, Lo YM, Johnson PJ. Silencing of GSTP1 gene by CpG island DNA hypermethylation in HBV-associated hepatocellular carcinomas. *Clin Cancer Res* 2002; **8**: 1087-1092 [PMID: 11948118]
87 **Anzola M**, Cuevas N, López-Martínez M, Saiz A, Burgos JJ, de Pancorbo MM. No association between GSTP1 gene aberrant promoter methylation and prognosis in surgically resected hepatocellular carcinoma patients from the Basque Country (Northern Spain). *Liver Int* 2003; **23**: 249-254 [PMID: 12895264]
88 **Okochi O**, Hibi K, Sakai M, Inoue S, Takeda S, Kaneko T, Nakao A. Methylation-mediated silencing of SOCS-1 gene

in hepatocellular carcinoma derived from cirrhosis. *Clin Cancer Res* 2003; **9**: 5295-5298 [PMID: 14614012]

89 **Schagdarsurengin U**, Wilkens L, Steinemann D, Flemming P, Kreipe HH, Pfeifer GP, Schlegelberger B, Dammann R. Frequent epigenetic inactivation of the RASSF1A gene in hepatocellular carcinoma. *Oncogene* 2003; **22**: 1866-1871 [PMID: 12660822 DOI: 10.1038/sj.onc.1206338]

90 **Zhong S**, Yeo W, Tang MW, Wong N, Lai PB, Johnson PJ. Intensive hypermethylation of the CpG island of Ras association domain family 1A in hepatitis B virus-associated hepatocellular carcinomas. *Clin Cancer Res* 2003; **9**: 3376-3382 [PMID: 12960125]

91 **Matsumura T**, Makino R, Mitamura K. Frequent down-regulation of E-cadherin by genetic and epigenetic changes in the malignant progression of hepatocellular carcinomas. *Clin Cancer Res* 2001; **7**: 594-599 [PMID: 11297254]

92 **Kwon GY**, Yoo BC, Koh KC, Cho JW, Park WS, Park CK. Promoter methylation of E-cadherin in hepatocellular carcinomas and dysplastic nodules. *J Korean Med Sci* 2005; **20**: 242-247 [PMID: 15831995]

93 **Jain S**, Chang TT, Hamilton JP, Lin SY, Lin YJ, Evans AA, Selaru FM, Lin PW, Chen SH, Block TM, Hu CT, Song W, Meltzer SJ, Su YH. Methylation of the CpG sites only on the sense strand of the APC gene is specific for hepatocellular carcinoma. *PLoS One* 2011; **6**: e26799 [PMID: 22073196 DOI: 10.1371/journal.pone.0026799]

94 **Tannapfel A**, Wittekind C. Genes involved in hepatocellular carcinoma: deregulation in cell cycling and apoptosis. *Virchows Arch* 2002; **440**: 345-352 [PMID: 11956813 DOI: 10.1007/s00428-002-0617-x]

95 **Kaneto H**, Sasaki S, Yamamoto H, Itoh F, Toyota M, Suzuki H, Ozeki I, Iwata N, Ohmura T, Satoh T, Karino Y, Satoh T, Toyota J, Satoh M, Endo T, Omata M, Imai K. Detection of hypermethylation of the p16(INK4A) gene promoter in chronic hepatitis and cirrhosis associated with hepatitis B or C virus. *Gut* 2001; **48**: 372-377 [PMID: 11171828]

96 **Nomoto S**, Kinoshita T, Kato K, Otani S, Kasuya H, Takeda S, Kanazumi N, Sugimoto H, Nakao A. Hypermethylation of multiple genes as clonal markers in multicentric hepatocellular carcinoma. *Br J Cancer* 2007; **97**: 1260-1265 [PMID: 17968429 DOI: 10.1038/sj.bjc.6604016]

97 **Huang L**, Li MX, Wang L, Li BK, Chen GH, He LR, Xu L, Yuan YF. Prognostic value of Wnt inhibitory factor-1 expression in hepatocellular carcinoma that is independent of gene methylation. *Tumour Biol* 2011; **32**: 233-240 [PMID: 21052890 DOI: 10.1007/s13277-010-0117-6]

98 **Matsukura S**, Soejima H, Nakagawachi T, Yakushiji H, Ogawa A, Fukuhara M, Miyazaki K, Nakabeppu Y, Sekiguchi M, Mukai T. CpG methylation of MGMT and hMLH1 promoter in hepatocellular carcinoma associated with hepatitis viral infection. *Br J Cancer* 2003; **88**: 521-529 [PMID: 12592365 DOI: 10.1038/sj.bjc.6600743]

99 **Zhang YJ**, Chen Y, Ahsan H, Lunn RM, Lee PH, Chen CJ, Santella RM. Inactivation of the DNA repair gene O6-methylguanine-DNA methyltransferase by promoter hypermethylation and its relationship to aflatoxin B1-DNA adducts and p53 mutation in hepatocellular carcinoma. *Int J Cancer* 2003; **103**: 440-444 [PMID: 12478658 DOI: 10.1002/ijc.10852]

100 **Sun FK**, Fan YC, Zhao J, Zhang F, Gao S, Zhao ZH, Sun Q, Wang K. Detection of TFPI2 methylation in the serum of hepatocellular carcinoma patients. *Dig Dis Sci* 2013; **58**: 1010-1015 [PMID: 23108564 DOI: 10.1007/s10620-012-2462-3]

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2 **Lin GZ**, Wang XZ, Wang P, Lin J, Yang FD. Immunologic effect of Jianpi Yishen decoction in treatment of Pixu-diarrhoea. *Shijie Huaren Xiaohua Zazhi* 1999; **7**: 285-287

In press

3 **Tian D**, Araki H, Stahl E, Bergelson J, Kreitman M. Signature of balancing selection in Arabidopsis. *Proc Natl Acad Sci USA* 2006; In press

Organization as author

4 **Diabetes Prevention Program Research Group**. Hypertension, insulin, and proinsulin in participants with impaired glucose tolerance. *Hypertension* 2002; **40**: 679-686 [PMID: 12411462 PMCID:2516377 DOI:10.1161/01.HYP.0000035706.28494.09]

Both personal authors and an organization as author

5 **Vallancien G**, Emberton M, Harving N, van Moorselaar RJ; Alf-One Study Group. Sexual dysfunction in 1, 274 European men suffering from lower urinary tract symptoms. *J Urol* 2003; **169**: 2257-2261 [PMID: 12771764 DOI:10.1097/01.ju.0000067940.76090.73]

No author given

6 21st century heart solution may have a sting in the tail. *BMJ* 2002; **325**: 184 [PMID: 12142303 DOI:10.1136/bmj.325.7357.184]

Volume with supplement

7 **Geraud G**, Spierings EL, Keywood C. Tolerability and safety of frovatriptan with short- and long-term use for treatment of migraine and in comparison with sumatriptan. *Headache* 2002; **42** Suppl 2: S93-99 [PMID: 12028325 DOI:10.1046/j.1526-4610.42.s2.7.x]

Issue with no volume

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No volume or issue

9 Outreach: Bringing HIV-positive individuals into care. *HRSA Careaction* 2002; 1-6 [PMID: 12154804]

Books

Personal author(s)

10 **Sherlock S**, Dooley J. Diseases of the liver and billiary system. 9th ed. Oxford: Blackwell Sci Pub, 1993: 258-296

Chapter in a book (list all authors)

11 **Lam SK**. Academic investigator's perspectives of medical treatment for peptic ulcer. In: Swabb EA, Azabo S. Ulcer disease: investigation and basis for therapy. New York: Marcel Dekker, 1991: 431-450

Author(s) and editor(s)

12 **Breedlove GK**, Schorfheide AM. Adolescent pregnancy. 2nd ed. Wieczorek RR, editor. White Plains (NY): March of Dimes Education Services, 2001: 20-34

Conference proceedings

13 **Harnden P**, Joffe JK, Jones WG, editors. Germ cell tumours V. Proceedings of the 5th Germ cell tumours Conference; 2001

Sep 13-15; Leeds, UK. New York: Springer, 2002: 30-56

Conference paper

14 **Christensen S**, Oppacher F. An analysis of Koza's computational effort statistic for genetic programming. In: Foster JA, Lutton E, Miller J, Ryan C, Tettamanzi AG, editors. Genetic programming. EuroGP 2002: Proceedings of the 5th European Conference on Genetic Programming; 2002 Apr 3-5; Kinsdale, Ireland. Berlin: Springer, 2002: 182-191

Electronic journal (list all authors)

15 Morse SS. Factors in the emergence of infectious diseases. Emerg Infect Dis serial online, 1995-01-03, cited 1996-06-05; 1(1): 24 screens. Available from: URL: http://www.cdc.gov/ncidod/eid/index.htm

Patent (list all authors)

16 **Pagedas AC**, inventor; Ancel Surgical R&D Inc., assignee. Flexible endoscopic grasping and cutting device and positioning tool assembly. United States patent US 20020103498. 2002 Aug 1

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