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Research Article

miRNA and tropism of human parvovirus B19

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ABSTRACT

Parvovirus B19 has an extreme tropism for human erythroid progenitors. Here we propose the hypothesis explaining the tropism of human parvovirus B19. Our speculations are based on experimental results related to the capsid proteins VP1 and VP2. These proteins were not detectable in nonpermissive cells in course of these experiments, although the corresponding mRNAs were synthesized. Our interpretation of these results is an inhibition of translation in nonpermissive cells by human miRNAs. We bring support to our hypothesis and propose detailed experimental procedure to test it.

1. Introduction

Parvovirus B19 (Anderson et al., 1983; Burton and Caul, 1988; Young and Brown, 2004) is pathogenic for humans and has a remarkable tropism for human erythroid progenitor cells (EPS). It was shown that aside from the cellular receptors required for virus entry into cells, successful accomplishment of the virus replication cycle is a key determinant of B19V tropism (Ozawa et al., 1988b; Liu et al., 1992; Wang et al., 1995). Nonstructural protein 1 (NS1) is produced in both permissive and nonpermissive cells but capsid protein synthesis appears to be restricted to permissive erythroid progenitors (Liu et al., 1992; Pallier et al., 1997). In nonpermissive cells, the capsid proteins (VP1 and VP2) are hardly detectable, although the corresponding mRNAs are synthesized (Pallier et al., 1997). Guan et al. (2009) hypothesized that translation of the capsid proteins either is inhibited in the cellular microenvironments of nonpermissive cell types (it may be also formulated as "absence of inhibition factors in permissive cells") or requires special cellular factors that are abundantly expressed in permissive cells.

A few explanations to the tropism of B19 appear in the literature: (i) an unusual promoter type of VP1 and VP2 (Wang et al., 1995), (ii) a sequence or a structure of the 3′ untranslated region of VP2 (Ozawa et al., 1988a; Pallier et al., 1997), (iii) an unusual RNA splicing pattern of viral mRNA (Brunstein et al., 2000), (iv) a block in full length transcript production in cells nonpermissive for B19 (Liu et al., 1992), and (v) a very recent explanation saying that a codon usage limitation is the reason to the tropism (Zhi et al., 2010). Zhi et al. (2010) reported cell type-specific expression of the B19V capsid genes and greatly increased B19V capsid protein production in nonpermissive cells by codon optimization. Moreover, they successfully generated B19 virus-like particles in nonpermissive cells by transient transfection of a plasmid carrying codon-optimized VP2 gene. Our hypothesis is related to the interpretation of the abovementioned results of Zhi et al. (2010) as inhibition of translation in nonpermissive cells by human miRNAs and absence of inhibition factors in permissive cells. However, neither the mechanism underlying the unique tropism of B19V genome replication in human EPCs, nor the cellular factors involved, have been identified (Chen et al., 2011). We propose our explanation to the tropism of B19 because, in our opinion, the above-mentioned theories are not sufficiently convincing.

We propose here a possible mechanism of the inhibition of translation of certain viral mRNAs in B19 nonpermissive cells that is related to the inhibition of translation initiation effected by miRNAs ((Mathonnet et al., 2007; Watanabe et al., 2007a) and references wherein). It is known that human miRNAs have a potential to regulate various human-infecting viruses via binding and down regulation of their target genes as a form of antiviral defense (Pedersen et al., 2007; Watanabe et al., 2007a; Mahajan et al., 2009); however, impact of human miRNAs on the regulation of translation of parvovirus B19 proteins is insufficiently

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Table 1
64 isolates of the human parvovirus B19 (the synonym Erythrovirus B19) and their accession numbers.

E U38546.1	E U38511.1	H/p – FoBe AY768535.1	E AF113323.1	H/p N8 AB030673.1	H/p – AN30 AB126264.1	H/p BN30.1 DQ408301.1	R0416 DQ234769.2
E U38518.1	E U38510.1	H/p – NAN AY504945.1	H/p – C39 from in vitro culture – FN598218.1	H/p – C39 from plasma FN598217.1	H/p Gh2768 NS1 – AY582124.2	H/p – BN32.2 DQ333427.1	H/p – KU1 FJ591158.1
E U38517.1	E U38509.1	H/p AB550331.1	H/p AN87 AB126271.1	H/p AN85 AB126270.1	H/p – AN23 AB126262.1	H/p BN33.2 DQ333428.1	Vn115 DQ357065.1
E U38516.1	E U38508.1	C39 DQ293995.2	H/p – Br543 AY647977.1	H/p – AN66 AB126269.1	H/p – P1 – FJ265736.1	H/p BN31.2 DQ333426.1	Vn147 DQ357064.1
E U38515.1	E U38507.1	H/p J35 – AY386330.1	H/p NC_000883.1	H/p – AN56 AB126268.1	H/p BN30.3 DQ408305.1	R0748 DQ234779.2	AnTo DQ225151.1
E U38514.1	E U38506.1	H/p Gh3051 AY582125.2	H/p – LaLi AY044266.2	H/p – AN41 AB126267.1	H/p BN60.3 DQ408304.1	R0693 DQ234778.2	OsFr DQ225150.1
E U38513.1	E U31358.1	H/p – AN28 AB126263.1	H/p – AB030694.1	H/p – AN40 AB126266.1	H/p BN59.3 DQ408303.1	D1599 DQ234775.2	SN807 DQ225149.1
E U38512.1	H/p – D91.1 AY083234.1	E strain HV AF162273.1	H/p – Mi AB030693.1	H/p – AN34 AB126265.1	H/p BN58.3 DQ408302.1	R0227 DQ234771.2	KyMa DQ225148.1

Abbreviations: E – Erythrovirus B19; H/p – Human parvovirus B19

known. miRNAs belong to a group of non-protein-coding small RNAs (Lee and Ambros, 2001; Ambros et al., 2003; Griffiths-Jones, 2004, 2006, 2010; Kozomara and Griffiths-Jones, 2011). They take part in important biological processes and negatively regulate gene expression at the posttranscriptional level (Bartel, 2004) through translational repression (Wightman et al., 1993; Mathonnet et al., 2007), direct mRNA cleavage (Hecht et al., 2005), or mRNA decay mediated by miRNAs deadenylation (Wu et al., 2006; Wu and Belasco, 2008). miRNAs are also involved in regulating stem-cell differentiation (Kanellopoulou et al., 2005), cell-lineage differentiation, and development (Naguibneva et al., 2006).

To check the hypothesis that inhibition of translation is caused by miRNAs we should demonstrate: (a) there are human miRNAs that bind to viral mRNA coding VP2 gene and silence it in nonpermissive cells; (b) these targets exist in all isolates of B19; (c) targets of these miRNAs are destroyed by codon optimization; and (d) the genes that these miRNAs have been originated from their introns are not expressed in permissive cells. By means of bioinformatics, we can check only points a, b, and c. We would check whether available data on human miRNAs and various isolates of B19 support the hypothesis.

2. Materials and methods

2.1. Data

Using NCBI resources 64 different coding sequences of B19 VP2 gene were found available at the time of writing this manuscript. Some isolates are named Erythrovirus B19, the others – Human parvovirus B19. The list of these sequences is in Table 1. All these coding sequences are equal in length and are very similar. The sequence (gi|291045153:3296–4960, B19 isolate F27) was used as a reference viral sequence and named further "normal". The natural sequences from different isolates were compared with a synthesized DNA sequence coding the same VP2 protein as the gi|291045153 sequence but with a different codon usage. This sequence that will be named further an "optimized" sequence was taken from (Zhi et al., 2010) and got its name because of the "optimized" codon usage.

Pre-miRNA sequences and miRNAs were received from the database miRBase ((Griffiths-Jones, 2006, 2010; Kozomara and Griffiths-Jones, 2011), http://mirbase.org).

2.2. Methods

The program miRNA-Finder (http://sites.google.com/site/malaheenee/software/mirna-finder) has been used for search pre-miRNA inside human genes. At present there are many computational methods for (Lewis et al., 2003; Rehmsmeier et al., 2004; Kruger and Rehmsmeier, 2006; Watanabe et al., 2006, 2007a,b; Creighton et al., 2008; Kim et al., 2009; Maragkakis et al., 2009a,b, 2011). For calculation of energy hybridization to individual target predictions was used software RNAhybrid ((Rehmsmeier et al., 2004; Kruger and Rehmsmeier, 2006) http://bibiserv.techfak.uni-bielefeld.de/rnahybrid/). The algorithmic core of program is one of variations of the classic RNA secondary structure prediction (Zuker, 1989a,b). RNAhybrid determines the most favorable hybridization site between two sequences miRNA–mRNA. This program allows choosing the characteristics of the hybridization sites search (Rehmsmeier et al., 2004). The length and position of seed can be defined (Kruger and Rehmsmeier, 2006). RNAhybrid uses thermodynamics-based algorithms as well as other techniques (DIANA-microT http://diana.cslab.ece.ntua.gr (Maragkakis et al., 2009b, 2011), PicTar http://pictar.mdc-berlin.de (Krek et al., 2005; Rajewsky et al., 2005; Rajewsky, 2006). Some of other programs use algorithms of seed complementarity (TargetScan (Lewis et al., 2003, 2005) http://targetscan.org), miRanda (John et al., 2004) http://www.microrna.org). Watson-Crick pairing of the mRNA target site with this seed region seems to be the most important factor for miRNA target prediction (Zhuo and Nikolaus, 2011). Apart from seed pairing, pairing with 3′ end of miRNAs also plays a role in target recognition (Bartel, 2009). Base pairing mRNA with 3′ region of the miRNA can also compensate for a mismatch in the seed region. It can supplement seed pairing and consequently improves specificity and affinity binding. However, these so called "3′-supplementary sites" are rare and only have weak effect (Bartel, 2009). Our script E-RNAhybrid (http://sites.google.com/site/malaheenee/software/) computes a score (ratio $\Delta G/\Delta G_m$), p-value. A ratio $\Delta G/\Delta G_m$ value (%), where ΔG_m equals binding energy for miRNA with perfectly complementary nucleotide sequence, is computed. Reliability degree (p-value) is estimated, that relies on ΔG and its standard deviation. Threshold significance is set to $p < 0.002$.

3. Results

64 isolate sequences of mRNA *VP2* gene of parvovirus B19 (Table 1) and the optimized variant of VP2 (Zhi et al., 2010) were aligned. The multiple alignments demonstrate that all isolates have identical length and that parsimonious explanation is that the only mutations are of "substitution" type and no indels happened (Fig. 1).

Instead of showing all 65 sequences of the performed multiple alignment only the "normal" sequence (gi|291045153:3296–4960)