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A single amino acid substitution in the mRNA capping enzyme λ2 of a mammalian orthoreovirus mutant increases interferon sensitivity

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ABSTRACT

In the last few years, the development of a plasmid-based reverse genetics system for mammalian reovirus has allowed the production and characterization of mutant viruses. This could be especially significant in the optimization of reovirus strains for virotherapeutic applications, either as gene vectors or oncolytic viruses. The genome of a mutant virus exhibiting increased sensitivity to interferon was completely sequenced and compared with its parental virus. Viruses corresponding to either the parental or mutant viruses were then rescued by reverse genetics and shown to exhibit the expected phenotypes. Systematic rescue of different viruses harboring either of the four parental genes in a mutant virus backbone, or reciprocally, indicated that a single amino acid substitution in one of λ2 methyltransferase domains is the major determinant of the difference in interferon sensitivity between these two viruses.

Introduction

Mammalian reoviruses are among a plethora of viruses that are presently under investigation for their ability to preferentially infect and destroy transformed/cancer cells. This has led to recent progress in their clinical use against various forms of human cancers (briefly reviewed among others by Kelly et al., 2009; Black and Morris, 2012). In contrast to most other "oncolytic" viruses presently under consideration for clinical use, reoviruses are naturally oncolytic and have not been submitted to genetic manipulation for their clinical use. In fact, it was not possible until recently (Kobayashi et al., 2007) to easily manipulate the reovirus genome using a plasmid-based reverse genetics system.

The initial observations that led to the utilization of reovirus as an "oncolytic" virus suggested that a decreased activity of the interferon-inducible double-stranded RNA-dependent protein kinase (PKR), which occurs upon Ras-induced cell transformation, was responsible for the preferential infection and destruction of cancer cells by reovirus (Strong et al., 1998). Despite the fact that multiple steps of the reovirus replicative cycle are likely involved in preferential replication and virus-induced cancer cell death (Lemay et al., 2007; Marcato et al., 2007), further work has supported the idea that a lower induction of interferon in Ras-transformed cells favors viral propagation and could be of major importance to the virus' oncolytic activity (Shmulevitz et al., 2010).

To further support the idea that interferon-induced antiviral pathways are critical in the ability of reovirus to discriminate between parental and Ras-transformed cells, a mutant virus obtained by chemical mutagenesis was selected for its increased sensitivity to interferon and was actually shown to exhibit a more complete blockage than the wild-type virus in parental cells, even at extremely high multiplicity of infection (Rudd and Lemay, 2005).

Multiple reovirus genes are suspected to be involved in the control of the interferon innate immune response (Sherry, 2009; Sherry et al., 2009). The μ2 protein was shown to be the major determining factor in the control of interferon signaling induction by altering the subcellular localization of IRF-9 (Irvin et al., 2012; Zurney et al., 2009). The σ3 protein could rather affect interferon sensitivity due to its ability to sequester the dsRNA activator of the interferon-inducible protein kinase PKR; however, this property of σ3 could be modulated by μ1 since the σ3-μ1 heterohexamer does not appear to bind dsRNA (Schiff, 2008). Finally, at least in some cell types, the σ2 and λ2 protein were previously suspected to play a role in the control of either induction of interferon signaling or virus' sensitivity to interferon (Sherry et al., 1998).

In order to clarify which genetic determinants are involved in the large difference in interferon sensitivity between the parental and previously identified P4L-12 mutant virus (Rudd and Lemay, 2005), the complete sequence of the viral genome was determined. Both viruses were then reconstructed by site-directed mutagenesis and reverse genetics, since a number of differences

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were observed between the isolate used to construct the reverse genetics plasmids and the parental laboratory stock initially used to isolate the mutant virus. Each four genes exhibiting differences between the parental and mutant viruses were then individually substituted either to replace the mutant gene by its parental counterpart or replacing the parental gene by its mutant counterpart in the parental background. This clearly demonstrated that the single amino acid substitution in one of the two methyltransferase domains of λ2 is the major determinant of the difference in interferon sensitivity between these two viruses.

Results

Sequencing of the interferon-sensitive P4L-12 mutant

In order to determine the differences in sequence between the interferon-sensitive P4L-12 mutant (Rudd and Lemay, 2005) and the original wild-type type 3 Dearing (T3D) (T3/Human/Ohio/Dearing/55) from which it was derived, the whole coding sequence of the genome was analyzed by RT-PCR on the semi-purified viral genome from both the mutant and wild-type laboratory stocks, followed by direct sequencing of the PCR products, as described in Section Materials and Methods and previously used in the laboratory (Jabre et al., 2013).

Despite the fact that the P4L-12 mutant was obtained following treatment with a high dose of the chemical mutagen nitrosoguanidine (Rudd and Lemay, 2005), only 4 genes were found to exhibit nonsynonymous mutations compared to the wild-type resulting in a total of 6 amino acids substitutions (Table 1). Of these, only the λ2 T636M, μ1 P315S and σ3 M221I are unique among currently available sequences of T3D (Table 2 and data not shown) and were thus of special interest. Six additional silent nucleotide substitutions were also found between the wild-type and mutant virus (GeneBank accession numbers KP208804 to KP208813 for the wild-type and KP208814 to KP208823 for P4L-12 mutant).

Sequence comparison of different stocks of wild-type reovirus type 3 Dearing

The complete sequences obtained were also compared with that of the wild-type virus encoded by the 10 plasmids of the reverse genetics system (Kobayashi et al., 2007) and at least one amino acid change between the two wild-type viruses was found in seven out of 11 proteins; the only completely identical proteins were σ2, σ3, σNS and σ1s. This is not the first time that such differences were observed between laboratory stocks of so-called reovirus type 3 Dearing (Coffey et al., 2006; Nygaard et al., 2013; Yin et al., 2004), this has even lead to the designation of subtypes such as $T3D^F$ (from Fields' laboratory), $T3D^N$ (from Nibert's laboratory) and $T3D^C$ (from Cashdollar's laboratory). Thus, for sake of simplicity, the laboratory virus stock used in the present study will be referred to as $T3D^S$ (Sandekian) while the wild-type virus rescued from the plasmids originally obtained from Kobayashi and Dermody will be referred to as $T3D^K$ (Kobayashi). In addition to the 12 amino acid substitutions, 11 silent nucleotide substitutions between the two viruses were also found (data not shown).

Comparative sequence analysis was also performed with the sequence of another complete T3D genome (referred to as $T3D^W$) that was used in a recent study by another group (van den Wollenberg et al., 2012) and the sequence of another laboratory stock deposited in the NCBI database by one further group (referred to as $T3D^H$); finally the sequence of the virus currently used in oncolytic virotherapy under the tradename "REOLYSIN®" was added to the comparison (Chakrabarty et al., 2014). Only complete sequences determined by direct sequencing of the viral genome were retained for this analysis since it is difficult to assume that sequences obtained earlier from cloned viral genes are necessarily representative and that they would actually generate a replication-competent virus when combined together.

The laboratory subtype, $T3D^S$, did not display any more differences from $T3D^K$ when compared to other completely sequenced genomes. While some positions are clearly more variable, all stocks showed the presence of 8 to 19 differences including 2–8

Table 1
Amino acids differences between wild-type reovirus type 3 Dearing laboratory stock ($T3D^S$) and derived mutant P4L-12.

	Wild-type ($T3D^S$)	Mutant (P4L-12)	Amino acid position
λ2	T	M	636
μ1	P	S	315
	T	A	449
μNS	V	A	705
σ3	G	E	198
	M	I	221

Table 2
Amino acids differences between different reovirus type 3 Dearing.

	$T3D^{K}$[a]	$T3D^{Sb}$[b]	$T3D^{W}$[c]	$T3D^{H}$[d]	Reolysin[e]	Amino acid position
λ3	M				L	979
	S				R	1045
	N	S	S		S	1048
	S		P			1186
λ2	G	E	E		E	504
	G				R	509
	I		M	M		1165
λ1	A		T			201
	I	S	S		S	500
	A		T			574
	Q				H	852
	M		V			982
	G		D			1017
μ2	A			V		122
	S	P	P		P	208
	R	Q	Q	Q	Q	342
	A				S	528
μ1	E				D	73
	T			I		142
	A	V	V			305
	–		R	R		439
	A		V	V		440
	Q		M	M		441
	A	T				449
	A		T			529
μNS	K	E	E		E	180
	A	V			V	705
	G				D	707
σ1	V				A	22
	T	I				249
	Y	S				253
	T	A	A		A	408
σ3	W				R	133
	G				K	198
	E		D	D	D	229

[a] T3D-Kobayashi, wild-type virus from the reverse genetics system (Kobayashi et al., 2007).
[b] T3D-Sandekian, original wild-type virus reported in this study
[c] T3D-Wollenberg, wild-type virus (R124) used in van den Wollenberg et al. (2012).
[d] T3D-Hosseini, wild-type unpublished virus sequence from NCBI database.
[e] T3D-Reolysin®, wild-type virus used in clinical trials as described in Chakrabarty et al. (2014).