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Short communication

Engineering human rhinovirus serotype-A1 as a vaccine vector

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ABSTRACT

Herein we describe the construction of recombinant human rhinoviruses (rHRVs) encoding HIV Gag or Tat by inserting the full length *tat* gene or regions of the *gag* gene flanked by sequences encoding the HRV 2A protease cleavage site into the junction between HRV genes encoding structural (P1) and non-structural (P2) proteins. Most recombinants were unstable, but this was corrected by mutation of the flanking cleavage sites. Thereafter, all rHRV constructs retained the inserts throughout six passages. Such constructs may find utility as vaccine vectors to generate mucosal immunity.

1. Introduction

HRVs are transmitted mucosally (Hayden, 2004), making them potential vectors for mucosally targeted human vaccines. However, these viruses have had limited development as vaccine vectors (Arnold et al., 2009; Smith et al., 1998) mainly due to concerns of pre-existing immunity to HRV in humans, lack of a suitable small animal model to test HRV-vectored vaccines, limited insertion capacity and poor genetic stability (Andino et al., 1994). However, a virus closely related to HRVs, viz. poliovirus, was used successfully as a vaccine vector to elicit protective immunity in macaques to challenge with SIVmac251 (Crotty et al., 2001). Moreover, vaccination of guinea pigs with recombinant HRV encoding the highly conserved membrane proximal external region ELDKWA epitope of HIV-1 induced broadly neutralising antibodies against several HIV pseudotypes (Arnold et al., 2009). Consequently, we wished to address the problems associated with HRVs as potential vaccine vectors. HRVs contain a 7.3 kb genome that encodes a 250 kDa polyprotein, which is cleaved by viral proteases $2A^{pro}$ and $3C^{pro}$ into precursor and mature proteins (Palmenberg et al., 2010). The HRV $2A^{pro}$ protease recognises and cleaves the amino acid sequence NTITTAG*PSDLY at the junction of the viral structural (P1) and non-structural proteins (P2/P3) (Palmenberg et al., 2010).

Minor group HRVs such as HRV-A1 replicate in murine (Tuthill et al., 2003) and human cells (Mosser et al., 2002) and infect mice and humans (Reithmayer et al., 2002), providing an opportunity to test HRV-vectored vaccines in a small animal model. HRV-A1 was reported some time ago to result in ~0.5% of HRV-related diseases (Monto et al., 1987) and this prevalence has been confirmed in more recent studies (Harvala et al., 2012; Martin et al., 2015). Furthermore, HVR-A1 is the rarest HRV serotype detected in humans (Gwaltney et al., 1968). Thus, it is unlikely that rHRV-A1, if used as a vaccine vector, will be significantly affected by pre-existing anti-HRV-A1 immunity. Furthermore, as there is no cross-neutralisation between different serotypes (Jacobs et al., 2013), HRV-A1 has the potential to deliver vaccines in humans who have been infected previously with other HRV serotypes. We thus describe the generation of a new replication-competent recombinant HRV-A1 for potential use as a vaccine vector.

2. Methods

2.1. Constructing rHRVs

To accommodate the limited packaging capacity of HRV, the HIV *gag* gene (Garrod et al., 2014) was divided into five discrete

Abbreviations: HIV, human immunodeficiency virus; AIDS, acquired immunodeficiency syndrome; HRVs, human rhinoviruses; wt-HRV-A1, wild type human rhinovirus serotype A1; rHRV-A1, recombinant human rhinovirus serotype-A1; CPE, cytopathic effect; UTR, untranslated region; NH-2, amino group; NIH, National Institutes of Health; M.O.I., multiplicity of infection.

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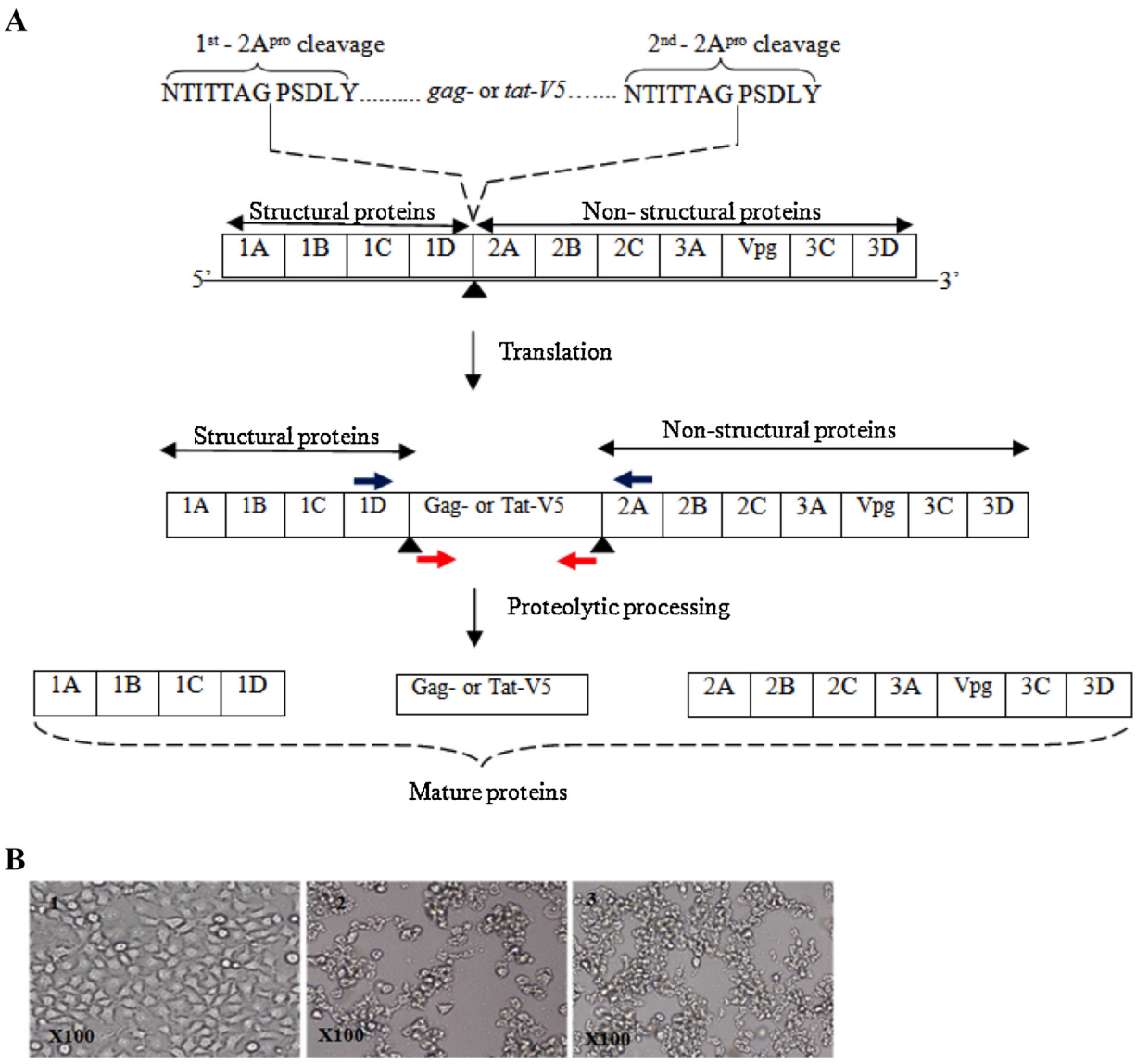

Fig. 1. Cloning strategy and production of replication-competent recombinant HRVs. (A) Schematic presentation of the construction of the replication-competent-recombinant HRV-A1 vector and expression strategy for the recombinant viral proteins. HRV genes and proteins are presented in boxes. $2A^{pro}$ cleavages at points indicated by solid triangles separates HRV structural from non-structural proteins and releases HIV proteins. Solid red arrows indicate binding sites for insert-specific primers and solid blue arrows indicate binding sites for primers that anneal to HRV-A1 sequences flanking the inserts. (B) Appearance of HI-HeLa cells 48 h post transfection with HRV RNA. 1, cell control (no virus or viral RNA); 2, wild type HRV and 3, rHRV gag-1. All other rHRVs showed similar effects in H1-HeLa cells.

overlapping fragments (*gag*-1 to *gag*-5), ranging from 393 to 513 bp. A sequence encoding a V5 tag (Garrod et al., 2014) was placed at the 3′ ends to facilitate detection of the truncated *gag* proteins, which contain few B cell epitopes (French et al., 2013). The *gag* fragments and the 303 bp full length *tat* gene (NIH AIDS Reagent Bank), flanked by the sequence encoding the viral $2A^{pro}$ cleavage site, were amplified by PCR. The PCR products, which contained Apa1 or Xho1 restriction enzyme cleavage sites on the 5′ or 3′ ends, and a cDNA plasmid encoding a replication-competent HRV 1A (Quiner and Jackson, 2010) were digested with the appropriate restriction enzymes, ligated (Fig. 1A) and transformed into *E.coli* (DH5-α), as described previously for a poliovirus vector (Andino et al., 1994). The resultant clones were sequenced to confirm the correct orientation of the respective inserts. The presence of the $2A^{pro}$ cleavage sites should result in release of the HIV proteins from the viral polyprotein via the action of the HRV-encoded protease. To generate genetically stable rHRV *gag-2* to *gag-5* and *tat*, we modified our cloning strategy by introducing several silent mutations into the coding sequences of both flanking $2A^{pro}$ cleavage sites, as described in results.

2.2. Virus culture

Full length viral RNA was generated using the MEGAScript T7 transcription kit (Life Technologies) and was used to transfect H1-HeLa cells using the TransIT®-mRNA transfection kit (Mirus) to produce replication-competent rHRVs. To confirm that the rHRVs retained the inserts through a series of passages, cDNA from total cellular RNA was extracted from virus-infected cells using the RNeasy-RT kit (Qiagen) and amplified by standard RT-PCR (KAPATaq, Biosystems) using (1) *gag* or *tat*-specific primers and (2) primers that annealed to HRV sequences flanking the insert (Fig. 1A). A complete list of all primers used in RT-PCR is presented in Fig. 1 in the supplementary data. The expression of Gag-1 was also confirmed in rHRV-*gag-1* by Western blot analysis using anti-V5 mouse monoclonal primary antibody (Life Technologies™)