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Molecular and functional interactions of cat APOBEC3 and feline foamy and immunodeficiency virus proteins: Different ways to counteract host-encoded restriction

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ABSTRACT

Defined host-encoded feline APOBEC3 (feA3) cytidine deaminases efficiently restrict the replication and spread of exogenous retroviruses like *Feline Immunodeficiency Virus* (FIV) and *Feline Foamy Virus* (FFV) which developed different feA3 counter-acting strategies. Here we characterize the molecular interaction of FFV proteins with the diverse feA3 proteins. The FFV accessory protein Bet is the virus-encoded defense factor which is shown here to bind all feA3 proteins independent of whether they restrict FFV, a feature shared with FIV Vif that induces degradation of all feA3s including those that do not inactivate FIV. In contrast, only some feA3 proteins bind to FFV Gag, a pattern that in part reflects the restriction pattern detected. Additionally, one-domain feA3 proteins can homo- and hetero-dimerize *in vitro*, but a trans-dominant phenotype of any of the low-activity feA3 forms on FFV restriction by one of the highly-active feA3Z2 proteins was not detectable.

Introduction

The replication of exogenous pathogens is often inhibited by specific cellular defense mechanisms, e.g. in the form of pathogen-specific restriction factors (Bieniasz, 2004). APOBEC3 (A3) cytidine deaminases have been recently shown to efficiently restrict the replication and spread of exogenous and endogenous retroviruses, retroid elements and other, distantly related viruses (Harris and Liddament, 2004). In face of this cellular restriction, retroviruses developed different, often highly specific strategies to counteract this restriction (Chiu and Greene, 2008; Cullen, 2006; Münk et al., 2010). The ongoing struggle of cellular defense and viral counter-action results in a dynamic co-evolution of the partners involved (LaRue et al., 2009). In cats, this resulted in a complex A3 locus that underwent a dramatic recent expansion resulting in three related feA3Z2 genes (feA3Z2a, -b, and -c) as well as one feA3Z3 gene, all encoding one-domain A3 proteins (Münk et al., 2008). In addition, a two-domain feA3Z2-Z3 cytidine deaminase is expressed by complex read-through transcription (Münk et al., 2008; Zielonka et al., 2010).

Identifying viral factors that shaped the cellular repertoire of A3 proteins and their evolution requires a comprehensive understanding of the viral factors targeted by the restriction system and those developed by the viruses to circumvent it. Recent studies on *Feline Immunodeficiency Virus* (FIV) presented evidence that the viral targets and counter-actions are highly related to that of *Human Immunodeficiency Virus* (Stern et al., 2010; Wang et al., 2011; Zielonka et al., 2010). However, little is known about other feline retroviruses that likely played also an important evolutionary role for the Felidae (Löchelt et al., 2005; Münk et al., 2008). In addition, this active co-evolution also leads to the establishment of cross-species transmission barriers since the viral counter-defense is in general ineffective or only partially active against heterologous restriction factors of non-authentic host species (Baumann, 2006; Münk et al., 2010; Zielonka et al., 2010).

Spumaretro- or foamy viruses (FVs) are a distinct group of retroviruses that are promising candidates for the development of novel viral vectors for gene delivery and vaccination (Rethwilm, 2007; Schwantes et al., 2003; Trobridge, 2009). Similar to the much better studied lentiviruses, FVs have a complex genetic makeup consisting of the canonical retroviral *gag*, *pol*, and *env* genes and the regulatory *bel 1*/*tas* gene encoding the viral transactivator (Linial, 1999; Rethwilm, 2010). In addition, the accessory *bet* gene that is generated via splicing counteracts cellular A3-mediated restriction and may have a role in particle release and in establishing viral persistence (Alke et al., 2001; Löchelt et al., 2005; Russell et al., 2005; Saib et al., 1995). In contrast to the unique

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molecular biology of FVs, insights into FV replication in the infected individual, the sites of FV replication, the authentic target cell(s), the potential disease association and the extent of virus replication during lifelong persistence are limited at present (Bastone et al., 2003; Linial, 2000; Saib, 2003). In this context, our studies on *Feline Foamy Virus* (FFV) replication in vitro but especially in cats with respect to host–virus interaction and interaction with other pathogens aim at enhancing understanding of these aspects of FV biology (Alke et al., 2000; Romen et al., 2006; Schwantes et al., 2003).

Different FVs of non-human primates have the capacity to cross species barriers to other primates and pose even a significant risk of zoonotic, inter-species transmission to humans (Heneine et al., 2003; Switzer et al., 2004). For instance, the prototypic/human FV isolate PFV has been shown to be the end-product of a zoonotic transfer of a chimpanzee FV to humans (Herchenröder et al., 1995). No disease has been associated with the limited number of well-documented zoonotic transmissions of FVs to humans (Khan, 2009). The apparent ease of interspecies transmissions of primate FV among primates implies that the active and extensive co-evolution of host-encoded restriction factors and the viral counter-defense have been insufficient to establish stable barriers towards cross-species transmission (Leendertz et al., 2008, 2010.). On the other hand, the control and containment of FV replication by different restriction systems may explain the apparent apathogenicity of FVs in their cognate as well as in their heterologous hosts (Bastone et al., 2003; Münk et al., 2010; Saib, 2003).

By comparing the APOBEC3-mediated restriction of FFV and FIV and the viral counter-defense via FFV Bet and FIV Vif, we showed that there are clear species-specific differences in APOBEC3-mediated restriction (Münk et al., 2008). Importantly, we show here that both FIV Vif and FFV Bet inactivate all known feline APOBEC3 proteins independent of whether they do or do not restrict the respective virus. Furthermore, Bet and Vif function by fundamentally different means: FIV Vif (like primate lentiviral Vif) leads to proteasomal degradation of all feA3 proteins while FFV Bet strongly binds all feA3s without decreasing their stability. This indicates that a Bet-mediated sequestration, masking, or the retention of feA3 proteins results in their functional inactivation. Finally, feA3 proteins bind to FFV Gag independent of whether they efficiently restrict FFV replication.

Results

Different A3 cytidine deaminases have been recently identified in the cat genome and their overall pattern of anti-retroviral activity against feline lenti-, onco-, and spumaviruses was defined: feA3Z3 and –Z2b-Z3 have only minor effects on FFV replication, the feA3Z2b and -c isoforms are strongly suppressive while feA3Z2a is less active (Münk et al., 2008). It was also shown that FFV Bet very efficiently counteracts feA3Z2b-mediated restriction by a unique mechanism independent of decreasing the stability of the restriction factor (Löchelt et al., 2005; Münk et al., 2008). Here we thoroughly determine the functional and physical interaction of the feA3s with proteins of FFV, the feline retrovirus that is strongly affected by these cellular restriction factors. In addition, we study functional and physical interactions of the different feA3 proteins.

Interaction of feA3 proteins and FFV Bet

We recently showed that FFV Bet specifically interacts with feA3Z2b, the feA3 form that is most potent in restricting FFV (Löchelt et al., 2005). We thus studied here whether Bet also binds the other feA3Z2 variants and/or those feA3 proteins that are not active against FFV (Münk et al., 2008). For this purpose, 293 T cells were co-transfected with either wt FFV clone pCF-7 or pCF-BetMCS, an FFV derivative encoding a full-length but non-functional Bet (Alke et al., 2001) and with plasmids encoding HA-tagged feA3Z2a, -b, -c forms (Fig. 1A) or feA3Z2b, -Z3, and –Z2b-Z3 (Fig. 1B). Cell lysates harvested 48 h post transfection (p.t.) were subjected to co-immuno-precipitation (co-IP) using an HA tag-specific monoclonal antibody. Subsequently, precipitated proteins were detected by immuno-blotting. While wt FFV Bet was efficiently co-precipitated by all three feA3Z2 iso-forms (Fig. 1A), the mutant BetMCS carrying heterologous amino acid sequences in a protein motif directly downstream of the Bel1/Tas coding sequence (Alke et al., 2001) was not precipitated at all (top panel) although feA3Z2a to -c expression levels were comparable (Fig. 1A, third panel from top). The result was not due to different steady-state

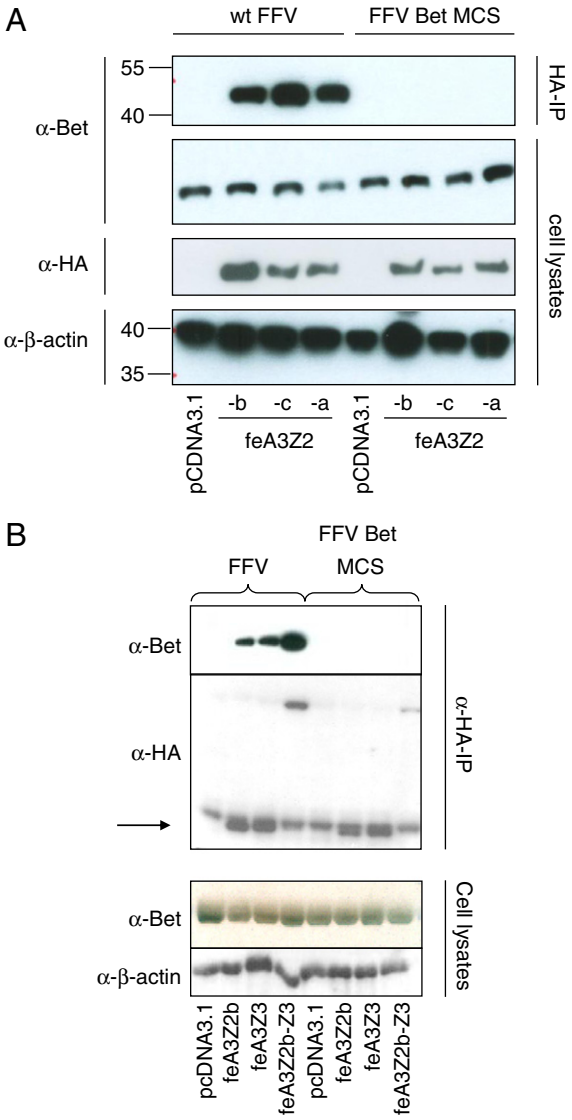

Fig. 1. The known feA3 proteins interact with wt but not with mutant FFV Bet. **A.** For co-IP experiments, 293 T cells were co-transfected with HA-tagged feA3Z2a, -b and -c expression plasmids and pcDNA3.1 control DNA together with wt pCF-7 and *bet* mutant pCF-BetMCSs. Cells were harvested 2 d p.t. and extracts used for co-IP with HA-specific antibodies. Co-precipitated proteins were detected by immuno-blotting. As shown in the top panel, only wt but not mutant Bet-MCS was co-precipitated. Below, immuno-assays of cell extracts document proper protein expression and identical loading. **B.** Similarly, HA-tagged feA3Z2b, -Z3 and -Z2b-Z3 expression plasmids and pcDNA3.1 control DNA together with wt pCF-7 and *bet* mutant pCF-BetMCS genomes were co-transfected into 293 T cells. Cell extracts were used for co-IP assays using HA-specific antibodies. As shown in the top two panels, only wt but not MCS-mutant Bet was co-precipitated with all different feA3 forms (the one-domain feA3Z2b and -Z3 forms marked by an arrow co-migrate with the IgG light chains used for co-precipitation which are also detected by the secondary antibody during immuno-blotting). Below, immuno-assays of cell extracts document proper expression of Bet and identical loading. In the panels, the source of antigen (from co-IPs or un-fractionated cell extracts), the antibody used for immuno-detection and the position of molecular mass markers are given.