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Research paper

Cloning, identification, and functional analysis of bone marrow stromal cell antigen-2 from sika deer (*Cervus nippon*)

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ABSTRACT

BST-2(tetherin/CD317/HM1.24) has been identified as a cellular antiviral factor that inhibits the release of a wide range of enveloped viruses from infected cells. Orthologs of BST-2 have been identified in several species including humans, monkeys, cows, sheep, pigs, and mice. In this study, we cloned the gene and characterized the protein of the BST-2 homolog from sika deer (*Cervus nippon*). cnBST-2 shares 37.8% and 74.2% identity with the BST-2 homologs from *Homo sapiens* and *Ovis aries*, respectively. The extracellular domain of cnBST-2 has two putative N-linked glycosylation sites and three potential dimerization sites. cnBST-2 was shown to be expressed on the cell surface, like human BST-2. Exogenous expression of cnBST-2 resulted in potent inhibition of HIV-1 particle release in 293T cells; however, this activity resisted antagonism by HIV-1 Vpu. Moreover, cnBST-2 was not able to activate nuclear factor-κB, in contrast to human BST-2. This study is the first report of the isolation and characterization of BST-2 from *C. nippon*.

1. Introduction

Interferon (IFN) system plays a key role in viral infection and replication *in vivo*. Four extensively studied proteins induced by type I IFN are the restriction factors apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G (APOBEC3G) (Sheehy et al., 2002), bone marrow stromal cell antigen 2 (BST-2) (Neil et al., 2008; Van Damme et al., 2008), tripartite motif 5-alpha (TRIM5α) (Stremlau et al., 2004) and SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1 (SAMHD1) (Goldstone et al., 2011). These host proteins are key players of the innate immune response and part of the first line of defense against lentiviruses. Viruses in turn have evolved to express adaptor molecules that antagonize these host cell restrictions, thereby allowing their replication to proceed efficiently (Sheehy et al., 2002; Stremlau et al., 2004; Lochelt et al., 2005; Jia et al., 2009).

BST-2 was first identified as a cellular restriction factor that blocks the release of human immunodeficiency virus type-1 (HIV-1) from infected cells. BST-2 exhibits broad antiviral activity against a wide range of enveloped viruses, including retroviruses (Jouvenet et al., 2009), alphaviruses (Jones et al., 2013; Mahauad-Fernandez et al., 2014), rhabdoviruses (Weidner et al., 2010; Sarojini et al., 2011), filoviruses (Kaletsky et al., 2009; Sakuma et al., 2009), flaviviruses (Pan et al., 2012; Pan et al., 2013), the hepatitis B virus (HBV) (Lv et al., 2015; Yan et al., 2015), and the influenza A virus (IAV) (Hu et al., 2017). Moreover, BST-2 might act as a viral sensor to stimulate a proinflammatory response (NF-κB activation) dependent upon an unknown step in the viral life cycle (Matsuda et al., 2003; Galao et al., 2012; Tokarev et al., 2013; Li et al., 2014). Different viral proteins antagonize BST-2, including HIV-1 Vpu, HIV-2/simian immune deficiency (SIV) envelope proteins, SIV Nef, Ebola glycoprotein, Kaposi's sarcoma-associated herpesvirus K5, HBV HBx, and IAV M2 (Sauter, 2014; Hu et al., 2017). The mechanisms of BST-2 antagonism by these viral proteins vary, and

Abbreviations: BST-2, bone marrow stromal cell antigen 2; IFNs, interferons; APOBEC3G, apolipoprotein B mRNA-editing enzyme, catalytic polypeptide-like 3G; TRIM5α, tripartite motif 5-alpha; SAMHD1, SAM and HD domain containing deoxynucleoside triphosphate triphosphohydrolase 1; HIV-1, human immunodeficiency virus type-1; HBV, hepatitis B virus; IAV, influenza A virus; SIV, simian immunodeficiency virus; KSHV, Kaposi's sarcoma-associsted herpesvirus; GPI, glycosyl-phosphatidylinositol; BLV, bovine leukemia virus; VSV, vesicular stomatitis virus; FIV, felien immunodeficiency virus; FBS, fetal bovine serum; PBMC, peripheral blood mononuclear cell; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; NC, nitrocellulose; BCIP, 5-bromo-4-chloro-3-indolylphosphate; NBT, nitro blue tetrazolium chloride; PBS, phosphate buffered solution; DAPI, 4′,6-diamidino-2-phenylindole; EGFP, Enhanced Green Fluorescent Protein; s-BST-2, shorter isoform of human BST-2; TGN, trans-Golgi-network

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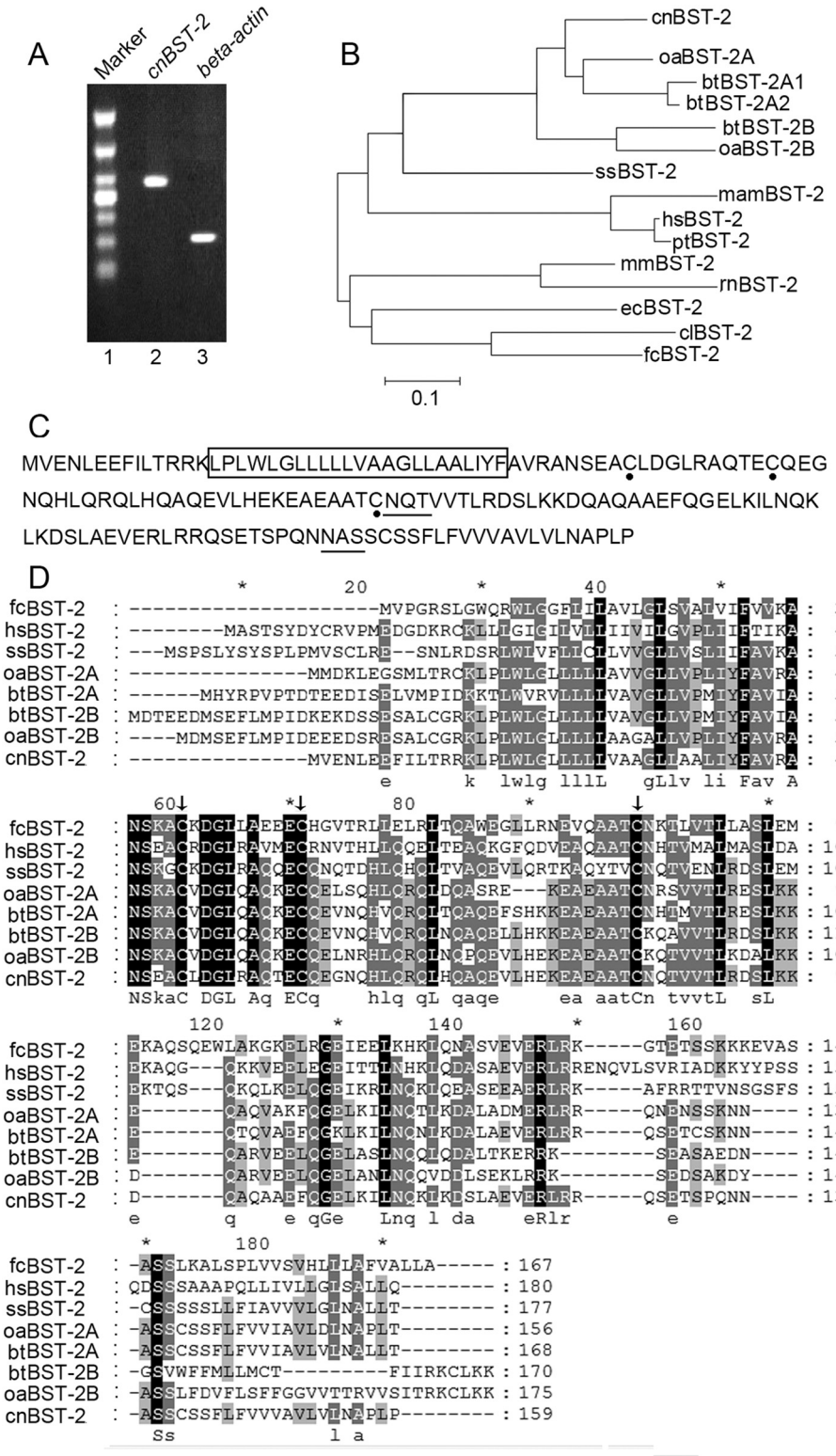

Fig. 1. Detection of *cnBST-2* transcription and amino acid sequence alignment. (A) PCR amplification of *cnBST-2* cDNA by RT-PCR from total mRNA of *C. nippon* PBMC cells. β-*actin* was used as a control. (B) Phylogenetic tree of BST-2 orthologs constructed using the neighbor-joining method. Each ortholog is differentiated using a letter in front of BST-2 as follows: cnBST-2, sika deer (*C. nippon*); oaBST-2, sheep, (*Ovis aries*); btBST-2, cows (*Bos taurus*); ssBST-2, pigs (*Sus scrofa*); mamBST-2, rhesus monkeys (*Macaca mulatta*); hsBST-2, humans (*Homo sapiens*); ptBST-2 chimpanzees (*Pan troglodytes troglodytes*); mmBST-2, mice (*Mus musculus*); rnBST-2, rats (*Rattus norvegicus*); ecBST-2, horses (*Equus caballus*); clBST-2, dogs (*Canis lupus familiaris*); and fcBST-2, cats (*Felis catus*). (C) Important functional domain and amino acid residue prediction for cnBST-2. The predicted transmembrane domain is boxed. Three Cys residues in the extracellular domain which are important for dimerization are shown with black spots. Two putative glycosylation sites are underlined. (D) Amino acid sequence alignment of BST-2 from *C. nippon* and other species. Three conservative Cys residues are indicated by the black arrows.

include protein trapping in intracellular compartments, proteasomal and lysosomal degradation, inhibition of BST-2 anterograde transport, inhibition of recycling, and other yet to be identified mechanisms (Sauter, 2014).

BST-2 is a type II single-pass transmembrane protein that shows varied expression among different cell types (Neil et al., 2008). It consists of a short N-terminal domain followed by an alpha-helical transmembrane domain, a labile coiled-coil ectodomain, and a C-terminal glycosylphosphatidylinositol anchor (Kupzig et al., 2003; Mahauad-Fernandez and Okeoma, 2016). The N-terminal intracellular domain of BST-2 contains an evolutionarily conserved tyrosine motif (YxY), which is implicated in nuclear factor (NF)-κB activation involving the recruitment of TAK1, Ubc13, TRAF2, and TRAF6 (Galao et al., 2012; Tokarev et al., 2013). Two potential N-linked glycosylation sites and three conserved cysteine residues are present in the extracellular domain, and are highly conserved at the same positions in humans,