Virology 444 (2013) 124–139

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/yviro

BST-2/tetherin is overexpressed in mammary gland and tumor tissues in MMTV-induced mammary cancer

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ARTICLE INFO

Article history:
Received 8 April 2013
Returned to author for revisions
3 May 2013
Accepted 31 May 2013
Available online 25 June 2013

Keywords:
BST-2
Tumor
Cancer
Epithelial cells
Mammary gland
Transformation
Tetherin
Human breast cancer
Mouse mammary tumor virus
PI3K
AKT
MIP1α
CCL3

ABSTRACT

BST-2 restricts MMTV replication, but once infection has established, MMTV modulates BST-2 levels. MMTV-directed BST-2 modulation is tissue-specific and dependent on infection and neoplastic transformation status of cells. In the lymphoid compartment of infected mice, BST-2 expression is first upregulated and then significantly downregulated regardless of absence or presence of mammary tumors. However, in mammary gland tissues, upregulation of BST-2 expression is dependent on the presence of mammary tumors and tumor tissues themselves have high BST-2 levels. Elevated BST-2 expression in these tissues is not attributable to IFN since levels of IFNα and IFNγ negatively correlate with BST-2. Importantly, soluble factors released by tumor cells suppress IFNα and IFNγ but induce BST-2. These data suggest that overexpression of BST-2 in carcinoma tissues could not be attributed to IFNs but to a yet to be determined factor that upregulates BST-2 once oncogenesis is initiated.

Introduction

Breast cancer is one of the leading causes of cancer associated deaths in women. Breast cancer related deaths are in part due to complications associated with metastasis that develops in regional lymph nodes and in distant organs, including bone, lung, liver, and brain (Fisher et al., 1983). The study of breast cancer biology and pathogenesis has greatly benefited from the use of mouse models with the inherent advantage of controlled experimentation. One of the leading breast cancer models is the mouse mammary tumor virus (MMTV) long terminal repeat (LTR)-driven models that permit controlled neoplastic transformation of mammary glands and eventual development of mammary cancer through targeted expression of various oncogenes or by infection with MMTV. MMTV is an endemic betaretrovirus that causes mammary carcinomas upon activation (Callahan and Smith, 2000; Nusse, 1991). Several mouse strains carry MMTV and transmit the virus via the germline or through the milk of infected females. MMTV initially infects antigen presenting cells (APCs), including dendritic cells and B cells followed by T cells (Courreges et al., 2007; Ross, 2010). MMTV-mediated neoplastic transformation of mammary glands and development of mammary cancer follows after APCs and T cells have been infected by MMTV and trafficked to mammary gland, infecting mammary epithelial cells (Ross, 2010). Induction of mammary tumors by MMTV is mediated by proviral integration into the host mammary epithelial cells and activation of protooncogenes, such as Wnt genes, Fgf family genes, Notch family genes, IntH/Int5, Int6, and Int41 (Callahan and Smith, 2000; Durgam and Tekmal, 1994; Garcia et al., 1986; Gray et al., 1986; Marchetti et al., 1995), and about 33 common insertion sites recently identified in a high-throughput MMTV insertional mutagenesis screen (Szabo et al., 2005).

Integration of MMTV into the host genome could result in over-expression of natural cellular proteins or genes that are otherwise tightly regulated. Like other cancers, breast cancer results from

Abbreviations: BST-2, Bone marrow stromal cell antigen 2; NMuMG, Normal murine mammary gland; FACS, Fluorescence activated cell sorting; PI3K, Phosphoinositide 3 kinase; MIP1-α/CCL3, Macrophage inflammatory protein 1-alpha

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http://dx.doi.org/10.1016/j.virol.2013.05.042

repression, loss of function, or overexpression of one or multiple genes. One of the genes whose expression accelerates mammary tumourigenesis induced by MMTV is Akt/PKB (Young et al., 2008). Akt is a multifaceted Serine/Threonin protein kinase involved in inhibition of apoptosis and the stimulation of cellular growth. There are 3 isoforms of Akt—named Akt1, Akt2, and Akt3 that are similar in their activation/phorsphorylation (Datta et al., 1999). Expression of different isoforms of Akt has been demonstrated in different human cancers, such as Akt1 expression in gastric and mammary cancers (Young et al., 2008, Maroulakou et al., 2007, Ju et al., 2007, Staal et al., 1977), Akt2 expression in ovarian and pancreatic cancers (Cheng et al., 1996, Bellacosa et al., 1995), and Akt3 in mammary and prostate cancer cell lines (Nakatani et al., 1999). The activation of Akt depends on activation of its upstream kinases, including Phosphatidylinositol 3-kinase (PI3K) (Datta et al., 1999). PI3K play important role in mediating cellular responses to extracellular signals (including cell survival, growth and migration) and are implicated in the progression of inflammatory diseases and cancer (Fougerat et al., 2009 and Denley et al., 2008). There are three distinct sub-groups of PI3Ks, namely, class I (A and B), class II, and class III; and the classification is based on their substrate specificity and sequence homology (Vanhaesebroeck et al., 2001). The class I PI3Ks consist of four catalytic isoforms, p110α, p110β and p110δ (class IA), and p110γ (class IB); each of which is bound to a regulatory subunit (p85α, p85β, p55γ, p55α, p50α for class IA; p101 or p84 for class IB) (Stephens et al., 1997, Krugmann et al., 1999, Suire et al., 2005, Voigt et al., 2006). The different PI3K isoforms interact with distinct subsets of downstream effectors thus allowing isoform specific roles, including enhancement of transcription of a diverse group of genes (Talapatra et al., 2001).

It has been shown that human cells expressing MMTV sequences have enhanced transcriptional profile of proinflammatory genes, such as tumor necrosis factor (TNF), transforming growth factor beta (TGF-β), and interferon-related genes with increased potential for cell growth (Fernandez-Cobo et al., 2006). Indeed, the transmembrane interferon-inducible gene called bone marrow stromal antigen 2 (BST-2) or tetherin and known for blocking the release of nascent enveloped viruses from the surface of infected cells (Chu et al., 2012; Dafa-Berger et al., 2012; Hammonds et al., 2012; Jones et al., 2013, 2012a; Kong et al., 2012; Laplana et al., 2013; Mangeat et al., 2012; Pillai et al., 2012), and inhibiting the replication of some retroviruses (Barrett et al., 2012; Jones et al., 2012a; Liberatore and Bieniasz, 2011) is overexpressed in different cancers. High levels of BST-2 were reported in multiple myelomas (Hundemer et al., 2006; Walter-Yohrling et al., 2003), metastatic ovarian cancers (Walter-Yohrling et al., 2003), neoplastic B cells (Goto et al., 1994), metastatic mammary cancers (Cai et al., 2009), and other cancer cells (Wainwright et al., 2011; Walter-Yohrling et al., 2003). Although these studies showed that BST-2 is overexpressed in cancer cells, there is no report on the progressive changes in BST-2 levels that occur in different tissues of an intact host during carcinogenesis. Here, we used C3H/HeN mouse strain to describe tissue-specific differences in the expression patterns of BST-2 in uninfected mice compared to mice suffering from MMTV infection and MMTV-induced carcinogenesis.

Results

Intrinsic expression of BST-2 in host cells potently impairs virus replication

Previously, we utilized BST-2 targeting siRNA to locally suppress BST-2 expression in murine lymph nodes and showed that down-regulation of BST-2 results in higher MMTV replication (Jones et al., 2012a). Since siRNA-dependent gene suppression is transient, we now use mice with different copies of the BST-2 gene (Fig. 1A) including homozygote WT (BST-2+/+; two copies of BST-2), homozygote knockout (BST-2−/−; no copy of BST-2), and the heterozygote (BST-2 +/−; one copy of BST-2) to probe the role of BST-2 in MMTV replication. Phenotypic examination of BST-2 transcript in naïve mice shows that, as expected, BST-2−/− mice have no BST-2 mRNA in the lymph node and spleen (Fig. 1B). However, mice with one copy of the BST-2 gene (BST-2+/−) express about half BST-2 mRNA compared to the WT mice (Fig. 1B). Similarly, BST-2 surface protein was higher in WT mice compared to their BST-2+/− counterpart (Fig. 1C). Since BST-2 mRNA is not present in BST-2−/− mice, we did not examine the surface protein. *Ex vivo* infection of lymphocytes and splenocytes obtained from WT, BST-2+/−, and BST-2−/− mice show that BST-2−/− mice have significantly higher MMTV proviral sequences as detected by real-time PCR amplification of the viral genome (Fig. 1D). Subcutaneous inoculation of MMTV into murine footpad tissues show that *in vivo*, MMTV replicates much better in BST-2−/− mice compared to BST-2+/−, and WT in that order (Fig. 1E). The spread of infection was also higher in BST-2−/− mice since more proviral sequence is detected in the spleen of BST-2−/− mice than BST-2+/− and WT mice (Fig. 1F). These data support our initial finding (Jones et al., 2012a) and reveal that loss of BST-2 predisposes mice to significant MMTV replication. Because expression of BST-2 significantly reduces viral load, we examined the impact of acute MMTV infection on BST-2 expression. Thus, we queried the level of BST-2 surface protein and mRNA in the lymph nodes and spleens of MMTV infected WT and BST-2+/− mice using FACS and RT-qPCR, respectively. We observed a substantial increase in BST-2 surface protein (Fig. 1G and H) and mRNA (Fig. 1I and J) day 1 post infection. This increase was followed by a significant decrease 7 days later (Fig. 1G–J). To determine whether higher viral products are released by infected BST-2−/− mice, we quantified plasma viral loads by measuring the level of cell-free plasma reverse transcriptase (RT) activity between WT, BST-2+/−, and BST-2−/− mice day 1 and 7 after infection. Virion-associated RT activity as determined by Molecular Probes EnzCheck Reverse Transcriptase assay was significantly higher in BST-2−/− mice compared to BST-2+/−, and WT mice in that order (Fig.1K).

Milk-borne infection of mice with MMTV results in changes in BST-2 expression

To further understand the effect of MMTV on BST-2 expression, we examined the levels of BST-2 expression in mice infected via the natural route. Spleens from age-matched pups nursed on C3H/HeN•MMTV− (naïve) and C3H/HeN•MMTV+ (infected) mice were examined for BST-2 expression using FACS analysis (Fig. 2A and B) and RT-qPCR (Fig. 2C). We observed that in the naïve mice, level of BST-2 protein and mRNA were lowest at day 3 and thereafter a steady increase up to day 30 (Fig. 2A and C). In contrast, BST-2 surface protein and mRNA levels were highest in milk-borne infected mice at day 3 with a significant decline on days 7, 21, and 30 (Fig. 2B and C). Examination of the proviral sequence by PCR reveals that naïve mice lack MMTV sequence as expected while infected mice harbor the proviral sequence (Fig. 2D). Of note, levels of proviral DNA were lower on day 3 compared to others days. This difference in the levels of proviral DNA could be attributed to the age of the animals. Furthermore, since level of BST-2 expression in MMTV infected mice decrease with age, we examined the level of BST-2 in age-matched naïve and milk-borne infected adult (5 weeks old) female mice. Results show that similar to the weanlings, levels of BST-2 protein and mRNA in infected mice are significantly lower compared to the level in their naïve counterparts (Fig. 2A–C). The suppression in BST-2 expression was observed with respect to BST-2 transcript (Fig. 2E) and surface protein (Fig. 2F) in peripheral blood mononuclear cells (PBMCs), spleens, and lymph nodes. As expected, the infected mice harbor MMTV proviral sequence in their chromosomes (Fig. 2G).