The Adaptor Protein p62 Mediates EBV LMP1 Signal Transduction

Ayrianna Sparks-Wallace
East Tennessee State University

Shunbin Ning
East Tennessee State University

The Adaptor Protein p62 Mediates EBV LMP1 Signal Transduction

Ayrianna Sparks-Wallace, Shunbin NING*

Center for Inflammation, Infectious Diseases and Immunity, Department of Internal Medicine, Quillen College of Medicine, East Tennessee State University, Johnson City, TN 37614, *Email: nings1@etsu.edu

ABSTRACT

p62 (also called SQSTM1, Sequestosome 1) is a ubiquitin sensor and a signal transducing adaptor that interacts with TRAF6 and facilitates the recruitment of ubiquitinated signal intermediators for the activation of NFκB in diverse contexts. In turn, p62 is induced by NFκB. However, the interaction between p62 and EBV latency has never been studied. We have recently published interesting and important results, which imply crucial roles of p62 in EBV latency. In this study, we first show that p62 is upregulated in EBV latency, depending on LMP1-mediated NFκB and AP1 activities, and in turn, p62 participates in LMP1 signal transduction through its interaction with TRAF6, promoting TRAF6 ubiquitination. shRNA-mediated p62 depletion downregulates LMP1-TRAF6 interaction and TRAF6 ubiquitination, and significantly impairs AP1 activity; however, with no detectable effects on NFκB activity. These observations imply that TRAF6-p62 interaction differentiates LMP1 signaling to NFκB and AP1 activation. As a consequence, p62 depletion promotes etoposide-induced apoptosis. These findings identify p62 as a novel player in EBV LMP1 signaling to AP1 activation that is crucial for LMP1-mediated ROS production.

Introduction

More than 50% of AIDS-related lymphomas (ARLs) are associated with Epstein-Barr Virus (EBV) infection. The World Health Organization (WHO) has defined EBV as a Class I carcinogen, and estimates that it causes greater than 200 thousand cases of cancer yearly. EBV also serves as a paradigm for the study of host-virus interactions. However, the mechanisms underlying EBV etiopathogenesis are not fully understood.

As the principal oncoprotein of EBV, Latent Membrane Protein 1 (LMP1) activates multiple transcription factors, including NFκB, AP1, and IRF7/IRF4, which promote cell survival and outgrowth. It is therefore vital to delineate the underlying mechanisms for understanding EBV-mediated oncogenesis.

p62 is a ubiquitin sensor and a signal transducing adaptor that interacts with TRAF6 and facilitates the recruitment of ubiquitinated signal intermediators for the activation of NFκB and AP1 in diverse contexts. In turn, p62 is induced by NFκB. However, the interaction between p62 and EBV latency has never been studied. We have recently published interesting and important results, which imply a crucial role for p62 in EBV-mediated oxidative stress.

RESULTS

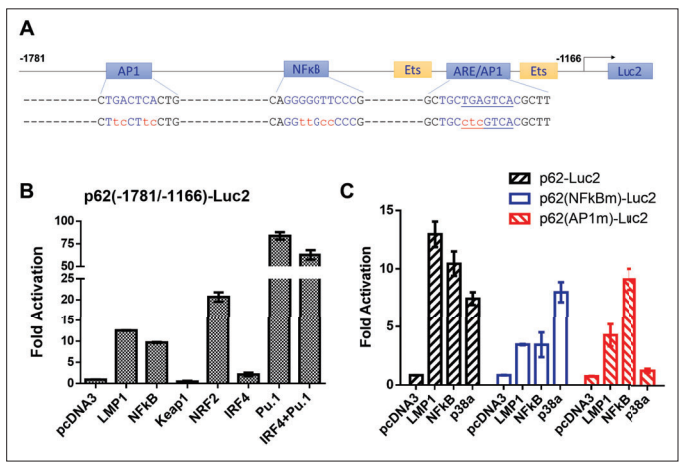

Fig 1. LMP1 activates p62 gene promoter

(A) A diagram showing the p62 promoter construct pGL3/p62(-1781/-1166)-Luc and its mutants. **(B)** LMP1, NFκB, NRF2, and Pu.1 transactivate the wild type pGL3/p62(-1781/-1166)-Luc. **(C)** Response of pGL3/p62(-1781/-1166)-Luc mutants to LMP1, NFκB, and p38α. 293 cells in 24-well plates were transfected with 150 ng IRF4, NRF2, or Pu.1, 150 ng p65 plus p50 (75 ng each), or 10 ng LMP1, 40 ng pGL3/p62(-1781/-1166)-Luc or its mutants, and 10 ng Renilla, for duplicates. Dual luciferase assay was performed 24 h after transfection. The ability of the vector control to activate the promoter construct was set to 1. At least 3 independent experiments were repeated, and representative results are shown

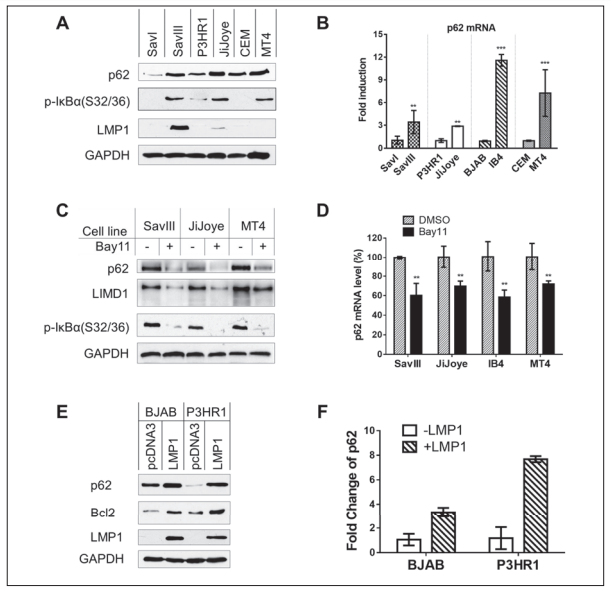

Fig 2. p62 is induced by NFκB/AP1 downstream of LMP1 signaling

(A)-(B) p62 expression levels are correlated with NFκB activity in paired B and T cell lines. RNA was extracted from indicated different pair of cell lines, and p62 mRNA expression level was evaluated by real-time qPCR. The average mRNA levels of the duplicates in SavI, BJAB, P3HR1, and CEM were set to 1. **(C)-(D)** Inhibition of NFκB activity in EBV-transformed cells downregulates p62 expression. NFκB activity in type 3 latency and MT4 cells was inhibited with the NFκB-specific inhibitor Bay11-7085 at the concentration of 2.5 μM for 48 h. The average mRNA levels of the duplicates in DMSO-treated cells were set to 100%. The p62 mRNA levels decreased by Bay11-7085 treatment are shown as percentage of those with corresponding DMSO controls. **(E)-(F)** LMP1 induces LIMD1 expression. BJAB and Akata stable cell lines expressing LMP1 or control were subjected to immunoblotting and qPCR analysis. The average mRNA levels of the duplicates in pcDNA3-transfected cells were set to 1. Statistical analysis was performed on results from three independent experiments

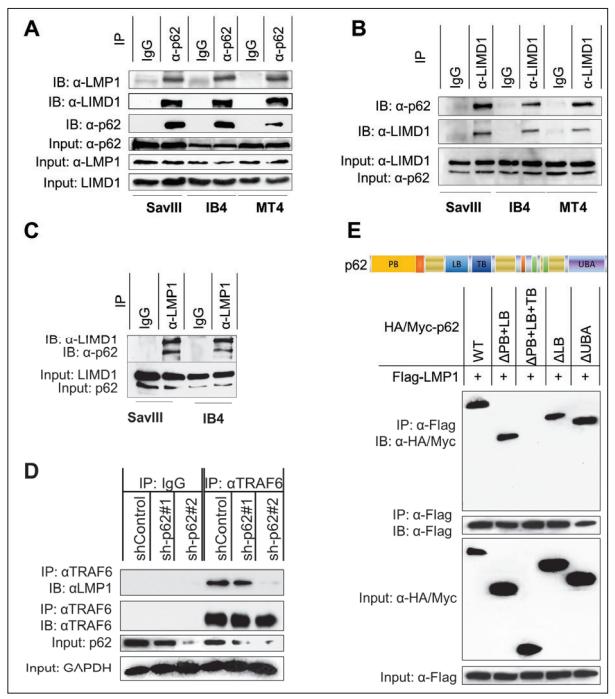

Fig 3. p62 interacts with TRAF6, LMP1 and LIMD1

(A)-(C) Endogenous p62, TRAF6, LMP1 and LIMD1 interact with each other in virus-transformed cells. Virus-transformed cells were subjected to IP and then IB with indicated mouse antibodies. **(D)** p62 depletion impairs LMP1-TRAF6 interaction. IB4 cells expressing control or p62 shRNAs were induced with doxycycline (1 μg/ml) for 3 days, and then subjected to IP with a rabbit TRAF6 antibody (Invitrogen), followed by IB with indicated antibodies. **(E)** The TRAF6-binding domain of p62 is required for its interaction with LMP1. 293T cells were transfected with Flag-LMP1 and HA- or Myc-tagged p62 (or its deletion mutants). Cells were collected 48 h after transfection and subjected to IP with Flag antibody (M2) and then IB with indicated antibodies.

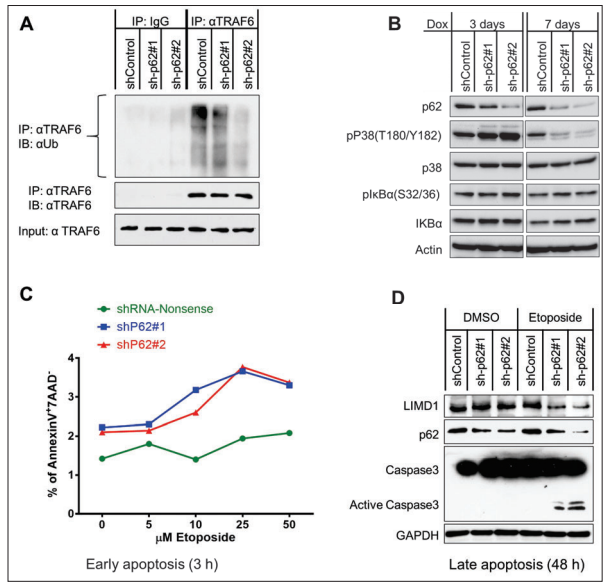

Fig 4. p62 promotes TRAF6 ubiquitination and AP1 activity in virus-transformed cells

(A) p62 depletion impair endogenous TRAF6 ubiquitination. IB4 cell lines stably expressing p62 shRNA #1 and #2 (or control shRNA) were induced by 1 μg/ml doxycycline for 48 h for shRNA expression, and cell lysates were then subjected to denaturing IP with a TRAF6 antibody (Invitrogen), followed by IB with indicated antibodies. **(B)** p62 depletion downregulates p38MAPK, but not NFκB activity. NFκB and p38 MAPK activities were evaluated using corresponding antibodies in IB4 stable cell line expressing p62 or control shRNAs, which were induced by for 1 μg/ml doxycycline 3 and 7 days**. (C)-(D)** p62 depletion potentiates etoposide-induced apoptosis. IB4 cell lines stably expressing p62 shRNA #1 and #2 (or control shRNA) were induced by 1 μg/ml doxycycline for 48 h, followed by treatment with different concentrations and time points of etoposide. Early and late apoptosis were evaluated, and representative results are shown.

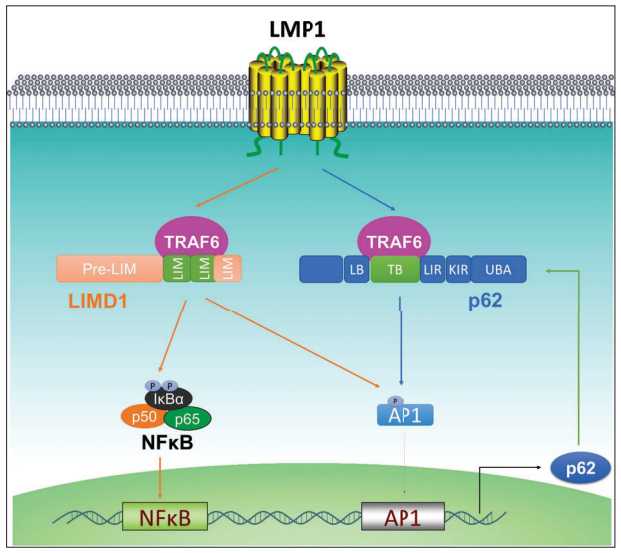

Fig 5. A diagram for the interplay between p62 and LMP1 signaling

LMP1 induces p62 expression through NFκB and AP1 pathways, In turn, p62 participates in LMP1 signal transduction by promoting TRAF6 ubiquitination through their interaction, and further promotes AP1 activation. Binding of LIMD1 to TRAF6 blocks LIMD1's ability to bind to p62. TRAF6 either promotes both NFκB and AP1 activation through interacting with LIMD1, or promotes AP1 activation through interacting with p62.

CONCLUSIONS

In this study, we show that p62 is upregulated in EBV latency, with the contribution of LMP1-mediated NFκB and AP1 activities. In turn, p62 participates in LMP1 signal transduction to AP1 activation through its interaction with TRAF6, promoting TRAF6 ubiquitination. As a consequence, p62 depletion promotes etoposide-induced apoptosis. These findings identify p62 as a novel player in EBV LMP1 signaling.

FUNDING SUPPORT

This work was supported by an NIH NIDCR grant to SN, and in part by the NIH grant C06RR0306551.