Molecular cloning, expression, overproduction and characterization of human TRAIP Leucine zipper protein

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ABSTRACT

The TRAIP interacting protein is known as a negative regulator of TNF-induced-nuclear factor, kappa-light-chain-enhancer of activated B cell (NF-kB) by direct interaction with the adaptor protein TRAF2, which inhibits the function of TRAF2 via the RINGCC domain protein. The TRAIP protein is composed of 469 amino acids with an N-terminal RING motif that is followed by a coiled coil (CC) and leucine zipper domain. TRAIP proteins are critical in programmed cell death, cell proliferation and differentiation, and embryonic development. The critical functions of TRAIP together with the molecular inhibitory mechanism effect of TRAIP have been reported by two different studies and have opened up new research into the field of TRAF biology. In this study, we designed different constructs of the Leucine zipper domain to find the over-expressed construct for further studies. We successfully cloned the C-terminal TRAIP containing the leucine zipper domain. In addition, we have over-expressed and purified the TRAIP LZ for their biochemical characterization.

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1. Introduction

The 53 KDa TRAF-interacting protein (TRAIP, TRIP, and RNF206) comprises an N-terminal RING finger, leucine zipper regions and coiled-coil that bind TRAF-family (Lee and Choi, 1997; Besse et al., 2007). The (TNF/TRAF--interacting protein (TRIP/TRAIP) from RING-type E3 ubiquitin ligase family undergoes autoubiquitination, however, in vivo, its substrate(s) has not been identified yet (Besse et al., 2007). Historically, TRAIP has been reported by many authors to interact with TNF/TRAIFs (Lee and Choi, 1997). TRAIP is expressed at low levels in most tissues (Chapard et al., 2012; Su et al., 2004), mainly found in the nucleolus of interphase mammalian cells (Zhou & Geahlen, 2009). It has been reported that NF-kB activation mediated by TRAF-2 is negatively regulated by a handful of binding partners including member of TRIP/TRAIP TRAF family associated with NF-kB activator (TANK/I-TRAF) proteins. Of all these binding partners, TRAIP plays main role in regulating the activation of NF-kB mediated by TRAF2. In Eukaryotics, several proteins are associated in regulation of cell cycle, thereby helping in producing daughter cell from parent cells (Karin and Gallagher, 2009). During S phase, DNA gets replicated and, during the final mitotic phase the chromosomes divide and shift toward poles, leads the division of parent cell into two daughter cells. Several modifications that regulate the progression of mitosis are controlled by, but not limited to ubiquitination, SUMOylation, and phosphorylation. (Wan et al., 2012; Hunt, 2013; Fournane et al., 2012; Kaseda et al., 2008). TRAIP along with TRAF signaling complex, plays an important role in TRAF2 mediated NF-kB activation (Boisvert et al., 2007; Karin and Gallagher, 2009). TRAF domain exhibits mushroom like trimeirc structure in solution (Kim et al., 2016). Recently, it was seen that an unknown protein shows ubiquitination when the BEN (ubiquitin–conjugating enzyme E2 N) interacts with the NOPO complex, and this mechanisms of association between proteins depicts genomic integrity in pre-mature
embryo of drosophila (Wallace et al., 2014). NOPO (No poles) defects was increased (Cheng and Baltimore, 1996). The current understanding related to recognition of TRAIP with TRAFs and associated diseases marks the TRAIP critical signaling molecule (Nasreena et al., 2019), and is tangled in different pathways related to signaling likewise RAP80 signaling pathway, DNA damage response, mitosis or cell cycle process, Syk-binding partner and also inhuman diseases (Bhat and Rather, 2018). Recently, the inhibitory molecular mechanism effect of TRAIP has been reported, the study showed that a TRAIP RINGCC hijacks the dimeric TRAF2CC domain. Moreover, it showed that a high oligomerized RING domain was required for the interface of the TRAF2CC domain as reported by Bhat et al. (2018). Here, we successfully cloned the human TRAIP protein containing the leucine zipper domain. Further, it was overexpressed and purified in two rapid steps by exploring Size exclusion chromatography (SEC) and Ni-NTA affinity chromatography. The main peak of TRAIP Leucine zipper domain eluted at 16 mL, which suggests that it forms the trimer, and was highly homogeneous as seen by SDS-PAGE, which was further confirmed by a Multi-Angle Light Scattering (MALS). The purity or homogeneity of protein was analyzed by SDS-PAGE.

2. Material and methods

2.1. TRAIP cloning, expression and purification

For polymerase chain reaction (PCR), CDNA of Human TRAIP (1–280 amino acids) was used as a template. Ndel and Xhol restriction enzymes (Enzynomics) were used to digest the PCR product. The pET24a plasmid was digested with the same enzymes. The generated construct leucine zipper corresponding amino acid 198–280, were then sub-cloned using plasmid vector pET24a purchased from Novagen (Daegu, South Korea) with C-terminal His-tag.

Table 1

| Name (LZ domain) | Species | Region | Amino acid | DNA | Enzyme | Vector | PCR | Cloning | Expression |
|------------------|---------|--------|------------|-----|--------|--------|-----|---------|------------|
| TRAIP-1 Human    | 186(D)-280(L) | 95 a.a | 285 bp | Ndel/Xhol | pET24a | Successful | Successful | Expression |
| TRAIP-2 Human    | 186(D)-270(E) | 91 a.a | 273 bp | Ndel/Xhol | pET24a | Successful | Successful | No expression |
| TRAIP-3 Human    | 186(D)-272(L) | 87 a.a | 261 bp | Ndel/Xhol | pET24a | Successful | Successful | No expression |
| TRAIP-4 Human    | 190 (G)-280(L) | 91 a.a | 170 bp | Ndel/Xhol | pET24a | Successful | Successful | No expression |
| TRAIP-5 Human    | 193(A)-280(L) | 88 a.a | 261 bp | Ndel/Xhol | pET24a | Successful | Successful | No expression |
| TRAIP-6 Human    | 190 (Q)-280(L) | 85 a.a | 252 bp | Ndel/Xhol | pET24a | Successful | Successful | No expression |
| TRAIP-7 Human    | 198 (A)-280(L) | 83 a.a | 246 bp | Ndel/Xhol | pET24a | Successful | Successful | Over-expression |
| TRAIP-8 Human    | 203(S)-280(L) | 78 a.a | 231 bp | Ndel/Xhol | pET24a | Successful | Successful | No expression |
The main peak fractions of the leucine zipper domain protein corresponding amino acid 198–280 was collected. Subsequently, each sample was centrifuged (10,000 \( \times \) g), at 4 °C for 10 min to remove the precipitate before loading on size exclusion chromatography column HR 10/30 (bed dimensions 10*300 mm) which was pre-equilibrated with solution containing 20 mM Tris-HCl at pH8.0 and 150 mM NaCl. In addition, the system was tied with three-angle light scattering refractive index detectors. After every 0.5 s, the date collected was analyzed by ASTRA program, suggesting molar mass plus mass distribution of each sample.

3. Results and discussion

The TRAIP (53 kDa) consists of 469 amino acids with N-terminal RING motif followed that coiled coil (CC) and leucine zipper (LZ) domain (Fig. 1). A human homologous TRAIP present in mice contained 470 amino acids and showed 76% sequence similarity. The N-terminal RING domain of human TRAIP possesses the E3 ubiquitin ligase activity. The C-terminal part of TRAIP (residues 211–470) has been involved to form a complex with CYLD and prevented the inhibitory activity of TRAIP (Regamey et al., 2003). For the in vitro biochemical characterization of TRAIP leucine, different constructs of TRAIP Leucine zipper domain were designed to find the best expression protein (Table 1). Among the constructs, only one construct was over-expressed which was used further in this study for biochemical characterization. The construct of the leucine zipper at the C-terminal end was eluted from rapid two-step chromatography, the affinity chromatography followed by the size exclusion chromatography. The sample from the Ni-affinity chromatography and gel filtration chromatography was pooled and analyzed by SDS-PAGE and the results are shown in (Fig. 2). In the figure, it is clear that the TRAIP Leucine zipper domain corresponding amino acid ([198–280]) (from lane E1-E5]) migrates to a position in the gel close to the 15 kDa band with a calculated molecular weight with His-tag (HHHHHH) of 10,561 Da. Furthermore, the stoichiometry changes were analyzed by MALS. The calculated molecular weight of the monomeric TRAIP-leucine zipper domain of TRAIP including the C-terminal his-tag were 10,561 Da, and the experimental molecular weights from MALS were 29,530 Da (2% fitting error), with a polydispersity of 1.04 for the TRAIP LZ domain (Fig. 3). Based on SEC and MALS results, TRAIP Leucine zipper is a trimer in solution. Further studies for understanding the in vivo molecular inhibitory mechanism will provide the answers to the unanswered questions in the field of TRAF-mediated biology.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Besse, A., Campos, A.D., Webster, W.K., Darnay, B.G., 2007. TRAF-interacting protein (TRIP) is a RING-dependent ubiquitin ligase. Biochem. Biophys. Res. Commun. 359, 660-664.
Bhat, E., Kim, C., Kim, S., Park, H., 2018. In vitro inhibitory mechanism effect of TRAIP on the function of TRAF2 revealed by characterization of interaction domains. Int. J. Mol. Sci. 19, 2457.

Bhat, E.A., Rather, I.A., 2018. A TRIP back in time to TRIP. J. Proteomics Bioinform. 11, 138–142.

Boisvert, F.M., van Koningsbruggen, S., Navascues, J., Lamond, A.I., 2007. The multifunctional nucleolus. Nat. Rev. Mol. Cell Biol. 8, 574–585.

Brummelkamp, T.R., Nijman, S.M., Dirac, Ä.M., Bernards, R., 2003. Loss of the cylindromatosis tumour suppressor inhibits apoptosis by activating NF-kappaB. Nature 424, 797–801.

Chapard, C., Hohl, D., Huber, M., 2012. The role of the TRAF-interacting protein in proliferation and differentiation. Exp. Dermatol. 21, 321–326.

Cheng, G., Baltimore, D., 1996. TANK, a co-inducer with TRAF2 of TNF- and CD40-mediated NF-kappaB activation. Genes Dev. 10, 963–973.

Fournane, S., Krupina, K., Kleiss, K., Sumara, I., 2012. Decoding ubiquitin for mitosis. Genes Cancer 3, 697e711.

Hunt, T., 2013. On the regulation of protein phosphatase 2A and its role in controlling entry into and exit from mitosis. Adv. Biol. Regul. 53, 173e178.

Karim, M., Gallagher, E., 2009. TNFR signaling: ubiquitin-conjugated TRAFic signals control stop-and-go for MAPK signaling complexes. Immunol. Rev. 228, 225–240.

Kaseda, K., Crevel, I., Hirose, K., Cross, R.A., 2008. Single-headed mode of kinesin-5. EMBO Rep. 9, 761e765.

Kim, C.M., Choi, J.Y., Bhat, E.A., Jeong, J.H., Son, Y.J., Kim, S., Park, H.H., 2016. Crystal structure of TRAF1 TRAF domain and its implications in the TRAF1-mediated intracellular signaling pathway. Sci. Rep. 6, 25526.

Lee, S.Y., Choi, Y., 1997. TRAF-interacting protein (TRIP); a novel component of the tumor necrosis factor receptor (TNFR)- and CD30-TRAF signaling complexes that inhibits TRAF2-mediated NFkappab activation. J. Exp. Med. 185, 1275–1285.

Nasreena, S., Mohammad, M.M., Johra, K., Irfan, A.R., Eijaz, A.B., 2019. Recognition of TRAIP with TRAFs: Current understanding and associated diseases. Int. J. Biochem. Cell Biol. 115. 105589.

Regamey, A., Hohl, D., Liu, J.W., Roger, T., Kogerman, P., Toftgard, R., Huber, M., 2003. The tumor suppressor CYLD interacts with TRIP and regulates negatively nuclear factor kappaB activation by tumor necrosis factor. J. Exp. Med. 15, 1959–1964.

Su, A.I. et al., 2004. A gene atlas of the mouse and human protein encoding transcriptomes. Proc. Natl. Acad. Sci. U. S. A. 101, 6062–6067.

Wallace, H.A. et al., 2014. TRIP/NOPO E3 ubiquitin ligase promotes ubiquitylation of DNA polymerase δ. Development 141, 1332–1341.

Zhou, Q., Geahlen, R.L., 2009. The protein-tyrosine kinase Syk interacts with TRAF-interacting protein TRIP in breast epithelial cells. Oncogene 28, 1348–1356.