Discovery of Myeloid zinc finger (MZF) 1 nuclear bodies

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Abstract

Myeloid zinc finger 1 (MZF1) is a multifaceted transcription factor that can act either as an transcriptional activator or a gene repressor. We examined its production of nuclear bodies (NBs) and subcellular localization. Proteomic and protein–protein interaction analysis were used to identify its cofactors and interactions. These revealed the presence of MZF1-NBs (intranuclear oligomers containing MZF1). MZF-NBs are similar to some other nuclear bodies, notably promyelocytic leukemia (PML) -NBs in terms of size and morphology. However the two structures appear to be different. MZF-NBs and PML-NBs were found to associate in the nucleus. Both MZF1 and PML are SUMO1-SUMOylated in PC-3 cells. Sumoylated MZF1 can interact with proteins containing SUMO-interaction motifs (SIM) through SUMO-SIM interaction. Interactome analysis revealed that its NBs participate in the stress response (TPR and UBAP2L), protein folding (CALR and ANKRD40), transcription, post-translational modification (TRIM33, ACOT7, CAMK2D, and CAMK2G), and RNA binding (ALURBP and CPSF5).

Keywords

Myeloid zinc finger 1; MZF1; nuclear body; PML; sumoylation; SCAN domain protein

Introduction

Myeloid zinc finger 1 (MZF1) is a multifaceted DNA-binding transcription factor that act as an transcriptional activator and a gene repressor. For instance MZF1 activates the *CDC37* [1], whereas this factor represses the *HSP90* [2]. Structurally, MZF1 comprises an N-terminal SCAN domain, a linker sequence, and C-terminal zinc finger motifs [3]. MZF1 forms oligomers and protein complexes with other SCAN-domain proteins via SCAN-SCAN interactions [2, 3]. Notable among these are SCAND1 and SCAN-D2 that upon binding can modify the transcriptional properties of MZF1 [2]. MZF1 and SCAND1 contain many phosphorylation, ubiquitination, and SUMOylation sites. Such post-translational modifications (PTMs) may play key roles in the protein-protein interactions that MZF1 participates in, beyond the SCAN-SCAN interaction.

This study identified the nuclear bodies (NBs, intranuclear oligimer) of MZF1. MZF-NBs were found to be similar to promyelocytic leukemia (PML) -NBs in terms of size and morphology, and to associate with them. The two structures are qualitatively different. Both MZF1 and PML were SUMO1-SUMOylated in PC-3 cells. SUMOylated MZF1 can interact with proteins containing SUMO-interaction motifs (SIMs) via the SUMO-SIM interactions. Using proteomics to screen for MZF1-interacting proteins, we identified 19 protein types that may interact with MZF-NBs.

MZF-NBs association with PML-NBs

MZF1 is a DNA-binding transcription factor that localizes in cell nuclei [1]. To further investigate its subcellular localization, we performed immunocytochemistry (ICC). This revealed that MZF1 formed large speckles, which we considered to be NBs, in the nuclei (Figure 1 A, B). MZF1 co-localized SCAND1, its cofactor. MZF-NBs and PML-NBs were found to associate in the nucleus (Figure 1C). These findings indicate the existence of MZF-NBs and reveal their association with PML-NBs.

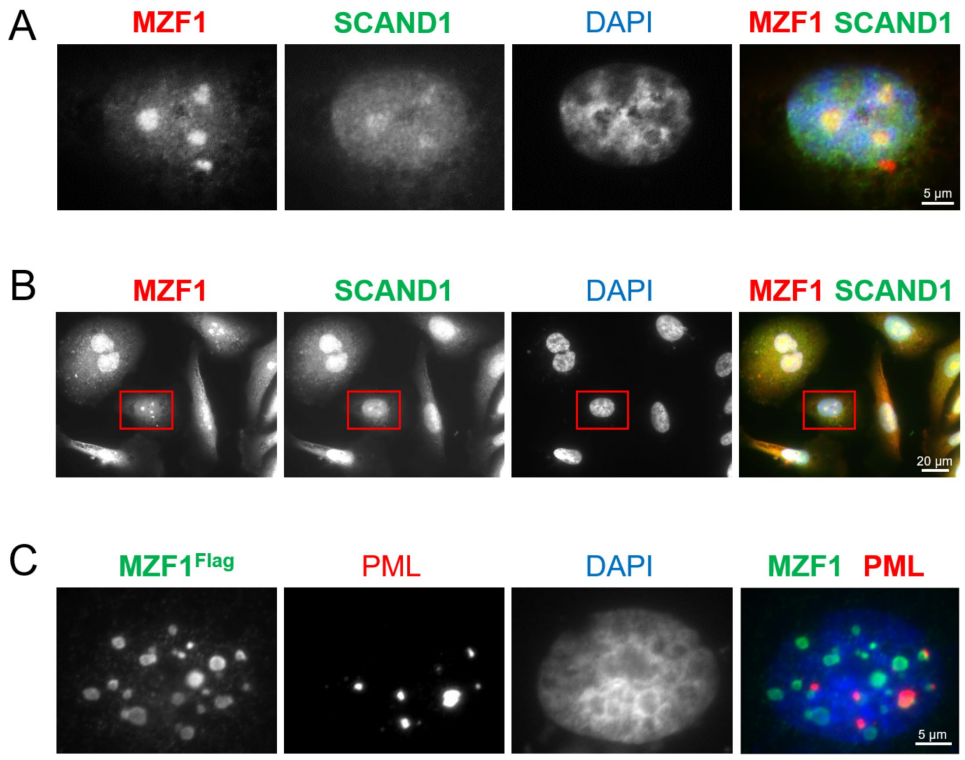

Figure 1. MZF-nuclear bodies (NBs) are associate with PML-NBs. (A, B) Endogenous MZF1 and SCAND1 were immuno-stained in PC-3 cells. MZF1 and SCAND1 colocalized in low- (A) and high- (B) magnification images. (C) Association between MZF-NBs and PML-NBs in the nucleus. MZF1-Flag was overexpressed in PC-3 cells.

SUMOylation sites and SIMs are abundant in MZF1

As nuclear body formation depends on post-translational modifications (PTM) [3], we predicted that MZF1, SCAND1, and SCAND2. Contain PTM sites. Five SUMOylation sites and two SUMO-interaction motifs (SIMs) were found in MZF1 (Figure 2), indicating that MZF-NBs form via oligomerization.

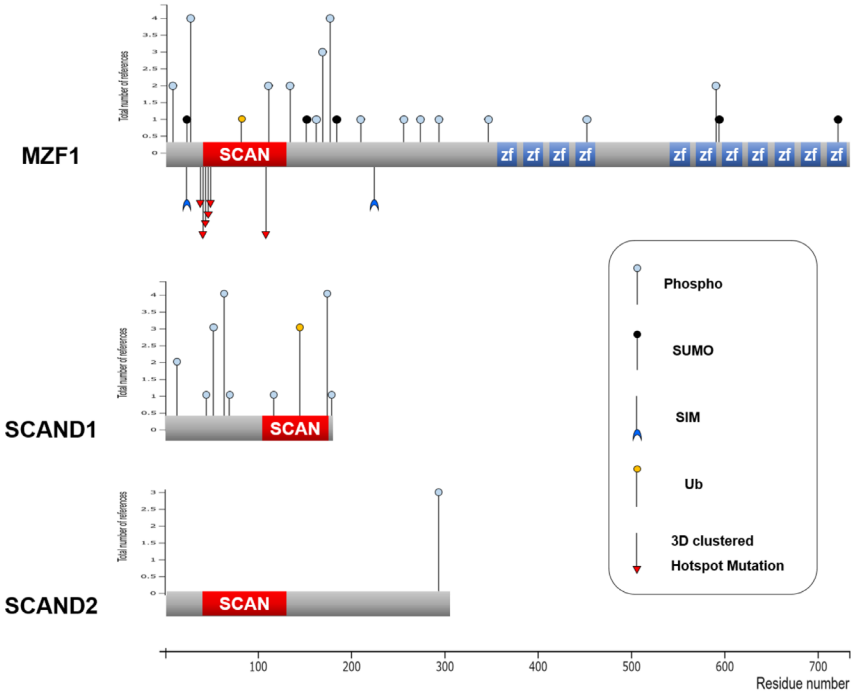

Figure 2. Domain structures of MZF1, SCAND1, and SCAND2 with post-translational modifications (PTMs). Red box, SCAN box. Blue box, zinc finger motif. Experimentally observed phosphorylation, sumoylation (SUMO), and ubiquitination (Ub) were referred from PhosphositePlus [4]. SUMO interaction motifs (SIM) were predicted [3]. 3D clustered hotspot mutations were found in the cBioPortal [5, 6].

Cell stress and culture conditions affect MZF1 PTM

To visualize PTMs, we performed western blotting analysis on cell extracts.

For MZF1, PC-3 cells exhibited an upward band shift for MZF1 relative to normal prostate epithelial cells (in which the native MZF1 has a molecular mass of 90 kD) (Figure 3A). Considering the molecular weight of the native MZF1, this shift could be due to di-SUMOylation. A similar upshift in MZF1 expression was observed in DU-145 cells overexpressing MZF1-Flag (Figure 3B). The further upshift of the MZF1 band indicates its poly-SUMOylation.

To investigate the effects of different media and types of cell stress on MZF1 PTM, we examined the effects of culture in mTeSR1 medium and KSFM medium and subjected the cells to heat-shock stress (43 °C for 30 min).

Both the mTeSR1-treated cells and those subjected to heat-shock stress exhibited an upshift for MZF1 (Figure 3C). It has been shown previously that the stress factor HSF1 becomes heavily SUMOylated upon heat shcok, indicating that this stress can trigger such modification of stress proteins [7, 8].

These results indicate that cell stress and culture conditions affect MZF1 PTM, potentially via SUMOylation.

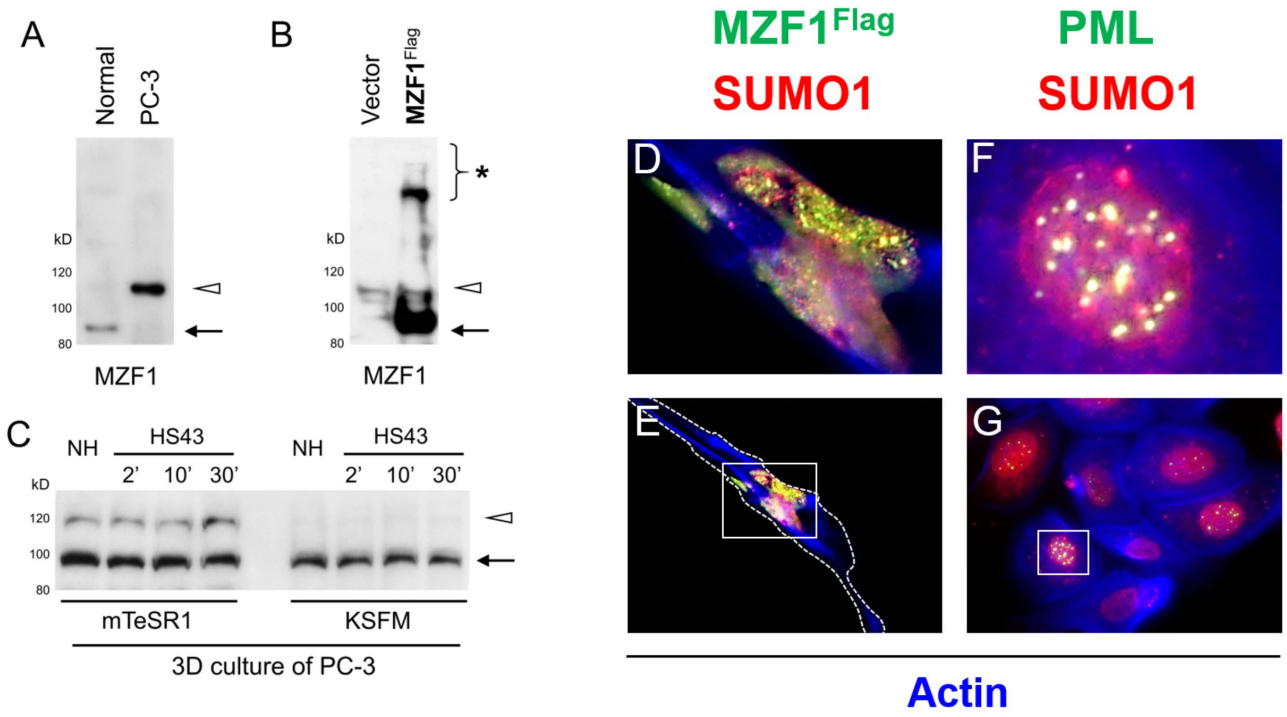

Figure 3. SUMO1 SUMOylation of MZF1 in PC-3 cells. (A–C) Western blotting of MZF1 in normal prostate cells and PC-3 cells (A), non-transfected and MZF1-Flag-transfected DU-145 cells (B), heat-shocked (HS) at 43 °C ("HS43") or untreated (no heat shock, "NH") PC-3 cells cultured in mTeSR1 or KSFM medium (C). Arrow, native full-length MZF1 (90 kD). Arrowhead, potential phosphorylation. White arrowhead, potential diSUMOylation. Asterisk, potential PTM. (D, E) Colocalization of MZF1-Flag (Green) and SUMO1 (Red). (F, G) Colocalization of PML and SUMO1 observed by immunofluorescence. Blue, actin stained by phalloidin.

MZF1 and PML were SUMO1-SUMOylated

To confirm the SUMOylation of MZF1, we performed ICC using a SUMO1 antibody. MZF1-Flag and SUMO1 were significantly co-localized, forming speckles, indicating SUMO1 SUMOylation of MZF1 (Figure 3D, E). Similarly, PML-NBs were modified by SUMO1 (Figure 3F, G). These results indicate that both MZF1 and PML were SUMO1-SUMOylated.

Interactome of MZF-NBs

To identify the protein contents of MZF-NBs, we performed Co-IP-LC-MS/MS. After immunoprecipitation of MZF1, we identified 510 interacting protein types (Figure 4A) and selected those with strong basal association or heat shock response. CAMK2D, ALURBP, and seven other protein types were strongly associated with MZF1 (Figure 4B). On the other hand, TPR, FKBP15, and the other nine others (including TPR and UBAP2L, stress response proteins) exhibited stress-inducible association with MZF1 (Figure 4C). The proteins strongly associated with MZF1 included RNA binding proteins (ALURBP and CPSF5), protein folding proteins (ANKRD40 and CALR), PTM regulators (ACOT7 and TRIM33) including kinases (CAMK2D and CAMK2G). STRING analysis revealed the protein-protein interaction network of all the MZF1 interactive proteins (Figure 4D).

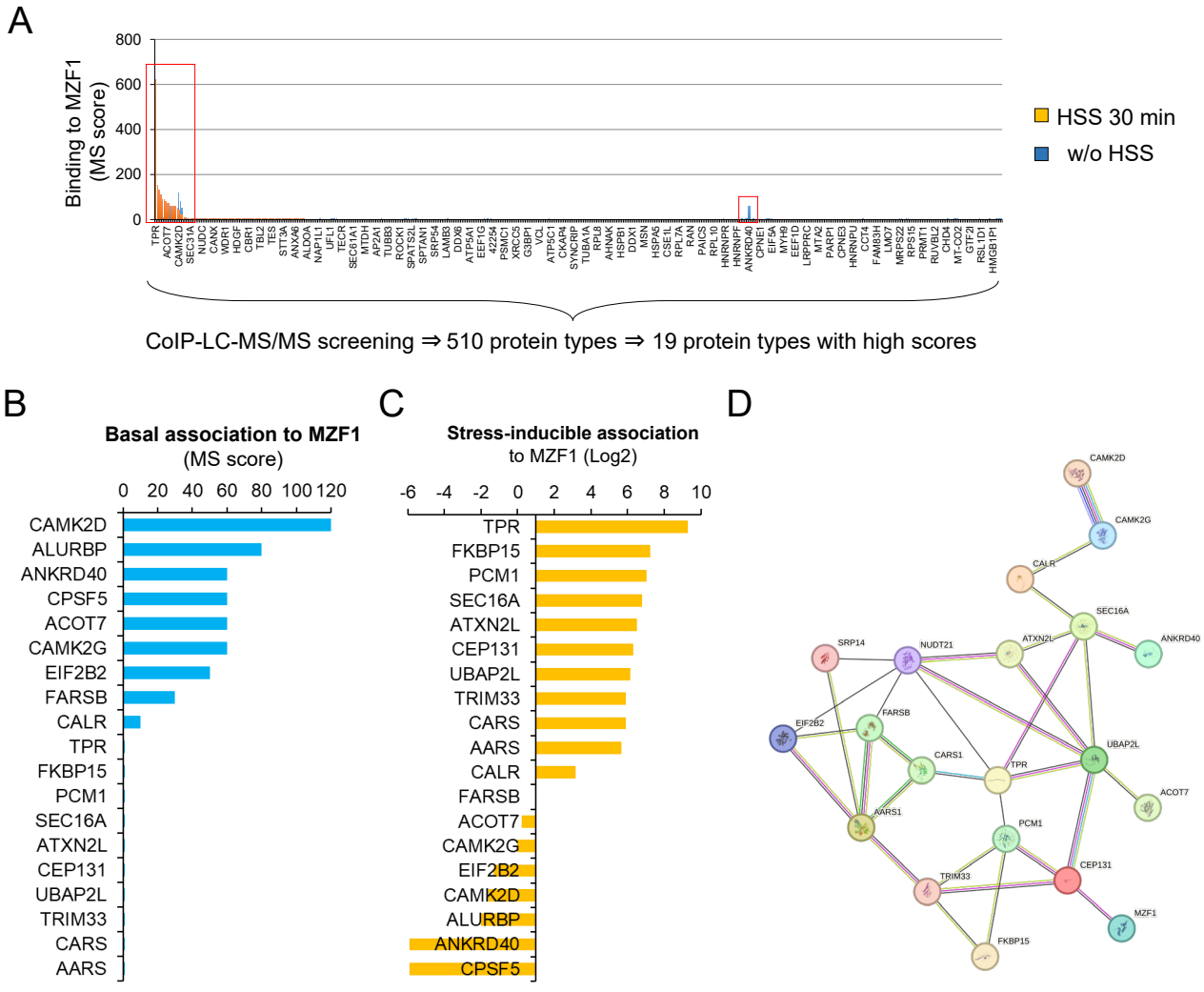

Figure 4. Proteomic analysis of MZF nuclear bodies (NBs). (A) Mass spectrometry (MS) scores based on CoIP-LC-MS/MS. PC-3 cells were treated with or without heat shock stress (HSS). Co-IP was performed using anti-MZF1 antibody. (B) Basal binding scores to MZF1. (C) Scores for stress-inducible binding to MZF1. (D) STRING analysis of MZF-NB.

Discussion

The multifaceted structure of MZF1 may be explained by its formation of NBs, which are larger than those of PML. NBs are assumed to perform various functions both within the nucleus and on its surface [9, 10]. Our screening identified various proteins involved in stress responses, protein folding, post-translational modifications, and RNA binding; this suggests that MZF-NB performs multiple functions through these proteins.

MZF-NBs were located close to, but not coincident with, PML-NBs. As MZF1 and PML have SUMOylation sites and SIMs, respectively, the SUMO-SIM interaction may have resulted in their close proximity, which may in turn cause events at the NB surface and the exchange of internal factors. It is notable that both heat shock factor 1 (HSF1) and MZF1 are both SUMOylated upon stress, suggesting that these events may be co-regulated and constitute part of the molecular chaperone stress response. Both factors are intimately involved in the regulation of molecular chaperone expression [1, 2, 11].

Although this screening did not reveal SCAND proteins, we were interested in two MZF1 binding factors, SCAND1 and SCAND2, which interact with SCAN domains [2, 12]. The various PTM sites and hotspots located around the SCAN domain are thought to alter the SCAN-SCAN interactions. However, we assume that the formation of MZF NBs involves multimerization via SCAN-SCAN and SUMO-SIM interactions.

These findings indicate that MZF-NBs are independent from those of PML. MZF1 has been shown to concentrate in PML-NBs [9, 13, 14] (0.2-1.0 microns in size), which are formed via oligomerization of PML and potentially >160 other protein components [15]. PML-NBs critically influence transcriptional activity and chromosomal structure by interacting with other factors [16].

The result of Fig. 1C is different from the MZF1/PML colocalization conclusion from a previous study [13]. In the two studies, experimental systems were different. Fig. 1C is overexpression of Flag-MZF1 and endogenous PML in castration-resistant prostate cancer cell line PC-3 cells. On the other hand, ref. 13 concluded that MZF1 is localized to PML-NBs. U2OS cells stably expressing GFP-PML were transfected with MZF1-BFP. Firstly, expression levels of PML are largely different in the two studies. Secondly, cell lines were different. Different cell lines should contain different signaling and PTM of both MZF1 and PML, resulting in different protein colocalization.

We confirmed the protein-protein interaction network by STRING analysis in Fig. 4D. Protein-protein interaction network was found in all the MZF1 interactive proteins. We then searched the functions of proteins and confirmed that TPR and UBAP2L are stress-response proteins. Moreover, ANKRD40 and CALR are protein-folding proteins, which also involve stress response. PTM regulators (ACOT7 and TRIM33) including kinases (CAMK2D and CAMK2G) were also identified reasonably. These facts confirmed the MZF1 interactive proteins which are involved in cell stress response. Our interactome analysis reveals that MZF-NBs participates in the stress response, protein folding, transcription, PTM, and RNA-binding.

Materials and methods

Cell culture

Castration-resistant prostate cancer cell line PC-3 and prostate adenocarcinoma cell line DU-145 were obtained from the American Type Culture Collection (ATCC, Manassas, VA). PC-3 cells were cultured in F-12K medium containing 10% fetal bovine serum (FBS), mTeSR1, or KSFM. DU-145 cells were cultured in DMEM supplemented with 10% FBS. Normal human prostate epithelial cells were purchased from Lonza (Basel,

Switzerland) and cultured in Prostate Epithelial Cell Basal Medium (Lonza) supplemented with bovine pituitary extract (BPE) medium, hydrocortisone, human epidermal growth factor (hEGF), epinephrine, transferrin, insulin, retinoic acid, triiodothyronine, and gentamicin sulfate–amphotericin (GA)-1000.

Plasmids

The plasmids pcDNA3.1-FLAG-MZF1 and pcDNA3.1 were used as described previously [1].

ICC and confocal laser scanning microscopy

ICC and confocal laser scanning microscopy were performed as described previously [1, 17]. Cells cultured in four-well chamber slides were fixed with 4% (w/v) paraformaldehyde in PBS for 15 min. The cells were permeabilized with 0.2% Triton X-100 in PBS for 15 min, then blocked in IHC/ICC blocking buffer high protein (eBioscience, San Diego, CA, USA) for 10 min and incubated with the following primary antibodies: anti-MZF1 (C10502; Assay Biotechnology, San Jose, CA), anti-SUMO1 (#4930; Cell Signaling Technology, Danvers, MA), anti-SCAND1 (ab64828; Abcam, Cambridge, UK), anti-PML (ab96051; Abcam), and anti-Flag M2 (F3165; Sigma-Aldrich, St Louis, MO). Alexa Fluor secondary antibodies (Thermo Fisher Scientific, Waltham, MA) were used in the blocking buffer. Between steps, the cells were washed twice with PBS for 5 min each time. The cells were mounted using ProLong Gold AntiFade Reagent (Thermo Fisher Scientific). Fluorescent images were captured using an AxioVision microscope equipped with an AxioCam MR3 microscope camera (Zeiss, Oberkochen, Germany).

PTM and mutations

Protein SUMOylation sites and SIMs were predicted as described previously [3]. Phosphorylation sites were predicted using PhosphoSitePlus. The 3D clustered hotspot mutations were identified using cBioPortal [5].

Western blotting

Western blotting was performed as described previously [1]. Anti-MZF1 (C10502; Assay Biotechnology) antibody was used.

Co-IP-LC-MS/MS

Co-immunoprecipitation (Co-IP) was performed using a Co-IP kit (Pierce, Thermo Fisher Scientific) and an anti-MZF1 antibody (C10502, Assay Biotechnology). Liquid chromatography–mass spectrometry (LC-MS/MS) was performed as previously described [18, 19].

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AUTHOR CONTRIBUTIONS

Conceptualization, Methodology, Investigation, Writing—original draft preparation, Visualization, Project administration: TE; Supervision, SKC; Funding acquisition, Resources: TE and SKC. Both authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

References

[1] T. Eguchi, T.L. Prince, M.T. Tran, C. Sogawa, B.J. Lang, S.K. Calderwood, MZF1 and SCAND1 Reciprocally Regulate CDC37 Gene Expression in Prostate Cancer, Cancers (Basel), 11 (2019) 792.
[2] M. Sheta, K. Yoshida, H. Kanemoto, S.K. Calderwood, T. Eguchi, Stress-Inducible SCAND Factors Suppress the Stress Response and Are Biomarkers for Enhanced Prognosis in Cancers, Int J Mol Sci, 24 (2023).
[3] T. Eguchi, T. Prince, B. Wegiel, S.K. Calderwood, Role and Regulation of Myeloid Zinc Finger Protein 1 in Cancer, J Cell Biochem, 116 (2015) 2146-2154.
[4] P.V. Hornbeck, J.M. Kornhauser, S. Tkachev, B. Zhang, E. Skrzypek, B. Murray, V. Latham, M. Sullivan, PhosphoSitePlus: a comprehensive resource for investigating the structure and function of experimentally determined post-translational modifications in man and mouse, Nucleic Acids Res, 40 (2012) D261-270.
[5] K. Ono, T. Eguchi, Large-Scale Databases and Portals on Cancer Genome to Analyze Chaperone Genes Correlated to Patient Prognosis, Methods Mol Biol, 2693 (2023) 293-306.
[6] E. Cerami, J. Gao, U. Dogrusoz, B.E. Gross, S.O. Sumer, B.A. Aksoy, A. Jacobsen, C.J. Byrne, M.L. Heuer, E. Larsson, Y. Antipin, B. Reva, A.P. Goldberg, C. Sander, N. Schultz, The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data, Cancer Discov, 2 (2012) 401-404.
[7] V. Hietakangas, J. Anckar, H.A. Blomster, M. Fujimoto, J.J. Palvimo, A. Nakai, L. Sistonen, PDSM, a motif for phosphorylation-dependent SUMO modification, Proc Natl Acad Sci U S A, 103 (2006) 45-50.
[8] J. Anckar, V. Hietakangas, K. Denessiouk, D.J. Thiele, M.S. Johnson, L. Sistonen, Inhibition of DNA binding by differential sumoylation of heat shock factors, Mol Cell Biol, 26 (2006) 955-964.
[9] M. Uggè, M. Simoni, C. Fracassi, R. Bernardi, PML isoforms: a molecular basis for PML pleiotropic functions, Trends Biochem Sci, 47 (2022) 609-619.
[10] U. Sahin, H. de The, V. Lallemand-Breitenbach, PML nuclear bodies: assembly and oxidative stress-sensitive sumoylation, Nucleus, 5 (2014) 499-507.
[11] T. Eguchi, C. Sogawa, K. Ono, M. Matsumoto, M.T. Tran, Y. Okusha, B.J. Lang, K. Okamoto, S.K. Calderwood, Cell Stress Induced Stressome Release Including Damaged Membrane Vesicles and Extracellular HSP90 by Prostate Cancer Cells, Cells, 9 (2020) 755.
[12] T. Eguchi, E. Csizmadia, H. Kawai, M. Sheta, K. Yoshida, T.L. Prince, B. Wegiel, S.K. Calderwood, SCAND1 Reverses Epithelial-to-Mesenchymal Transition (EMT) and Suppresses Prostate Cancer Growth and Migration, Cells, 11 (2022).
[13] L. Noll, F.C. Peterson, P.L. Hayes, B.F. Volkman, T. Sander, Heterodimer formation of the myeloid zinc finger 1 SCAN domain and association with promyelocytic leukemia nuclear bodies, Leuk Res, 32 (2008) 1582-1592.
[14] P. Heun, SUMOrganization of the nucleus, Curr Opin Cell Biol, 19 (2007) 350-355.
[15] E. Van Damme, K. Laukens, T.H. Dang, X. Van Ostade, A manually curated network of the PML nuclear body interactome reveals an important role for PML-NBs in SUMOylation dynamics, Int J Biol Sci, 6 (2010) 51-67.

[16] G. Dellaire, D.P. Bazett-Jones, PML nuclear bodies: dynamic sensors of DNA damage and cellular stress, Bioessays, 26 (2004) 963-977.
[17] T. Eguchi, S. Kubota, K. Kawata, Y. Mukudai, J. Uehara, T. Ohgawara, S. Ibaragi, A. Sasaki, T. Kuboki, M. Takigawa, Novel transcription-factor-like function of human matrix metalloproteinase 3 regulating the CTGF/CCN2 gene, Mol Cell Biol, 28 (2008) 2391-2413.
[18] K. Ono, T. Eguchi, Proteomic Profiling of the Extracellular Vesicle Chaperone in Cancer, Methods Mol Biol, 2693 (2023) 233-249.
[19] K. Ono, T. Eguchi, C. Sogawa, S.K. Calderwood, J. Futagawa, T. Kasai, M. Seno, K. Okamoto, A. Sasaki, K. Kozaki, HSP-enriched properties of extracellular vesicles involve survival of metastatic oral cancer cells, J Cell Biochem, 119 (2018) 7350-7362.