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RAPID COMMUNICATION

MZF1 (Myeloid Zinc Finger 1) activates transcriptional activity of p53 and suppresses breast cancer cell proliferation via acetyltransferase PCAF (P300/CBP associated factor)

p53 is an important tumor suppressor gene. The p53 pathway is activated in response to cellular stress stimulation. However, in more than 50% of breast cancers, *p53* is mutated or inactivated, which permits cancer growth.[1] Although a large number of co-regulators of p53 have been identified, the integrated molecular network of p53 and the key co-factors that could transactivate p53 remain unclear.[2] Myeloid Zinc Finger 1 (MZF1), a member of the SCAN-ZFP (SCAN-Zinc finger protein) family, has been involved in the occurrence and development of various types of malignant tumors, including breast cancer.[3] However, the exact mechanism of action remains unclear. Our study aims to investigate the cross-regulatory loop between MZF1 and p53 in breast cancer cells.

In clinical data, we found that RNA expression of *MZF1* was significantly down-regulated in breast cancer tissues compared with normal tissues (Fig. 1A; Fig. S1A). Next, we analyzed the protein level of *MZF1* in various cell lines. The highest expression of MZF1 was found in MCF10A cells, followed by T47D (p53 L194F), ZR-75-30, MCF-7, and MDA-MB-231 (p53 R280K) cells (Fig. 1B; Fig. S1B). At present, a lack of direct evidence and detailed mechanism of MZF1 on the proliferation of breast cancer cells. Hence, we examined the proliferation effect of MZF1 *in vitro*. We found that overexpression of Flag-MZF1 repressed the growth of MCF-7 cells, while *MZF1* knockdown enhanced the ZR-75-30 cell viability, and *vice versa* (Fig. 1C; Fig. S1C). However, MZF1 did not affect the proliferation of T47D or MDA-MB-231 cells (Fig. S1C). Moreover, the proportion of MCF-7 cells was arrested at the G0/G1 phase with ectopic expression of MZF1, whereas the T47D cells were not affected (Fig. 1D; Fig. S1D–F). Through the presented results, we inferred that MZF1 may inhibit the proliferation of breast cancer cells with wild-type p53 other than p53-mutated breast cancer cells. Hence, MCF-7 and ZR-75-30 cells were selected for further experiments.

MZF1 was statistically enriched in the vicinity of the p53-binding sites.[2] Moreover, the existence of interaction between MZF1 and p53 was found (Fig. 1E; Fig. S2A–C). To further prove this assumption, the GFP-MZF1 and Flag-p53 were both located in the nucleus of MCF-7 cells (Fig. 1F). Consistently, this suggests a direct interaction between MZF1 and p53 (Fig. 1G; Fig. S2D). Likewise, the C-terminal basic domain of p53 and the conserved acidic SCAN domain of MZF1 are required for their physical interaction (Fig. S2F, G).

Therefore, we investigated the effect of the interaction between p53 and MZF1 on the transactivation of p53. The overexpression of MZF1 enhanced the transcriptional activity of p53 in a dose-dependent manner, and *vice versa* (Fig. 1H; Fig. S3A, B). In order to further explore the effect of MZF1 on p53 transactivation dependent on p53, the activation effect of MZF1 was diminished when p53 expression was inhibited (Fig. 1I). Moreover, the transcriptional activity of p53 was enhanced by the overexpression of MZF1 and MZF1-Δ1, while was not affected by MZF1-Δ2 or -Δ3 (Fig. S3C). Besides, we found that MZF1-Δ1 could repress the growth of MCF-7 cells (Fig. S3D). Furthermore, MZF1 overexpression increased the mRNA level of *p21* (downstream target gene of p53), and *vice versa*. However, MZF1 did not have a distinct effect on *p53* (Fig. 1J; Fig. S3G). Similar results were obtained from its protein level (Fig. S3E–H).

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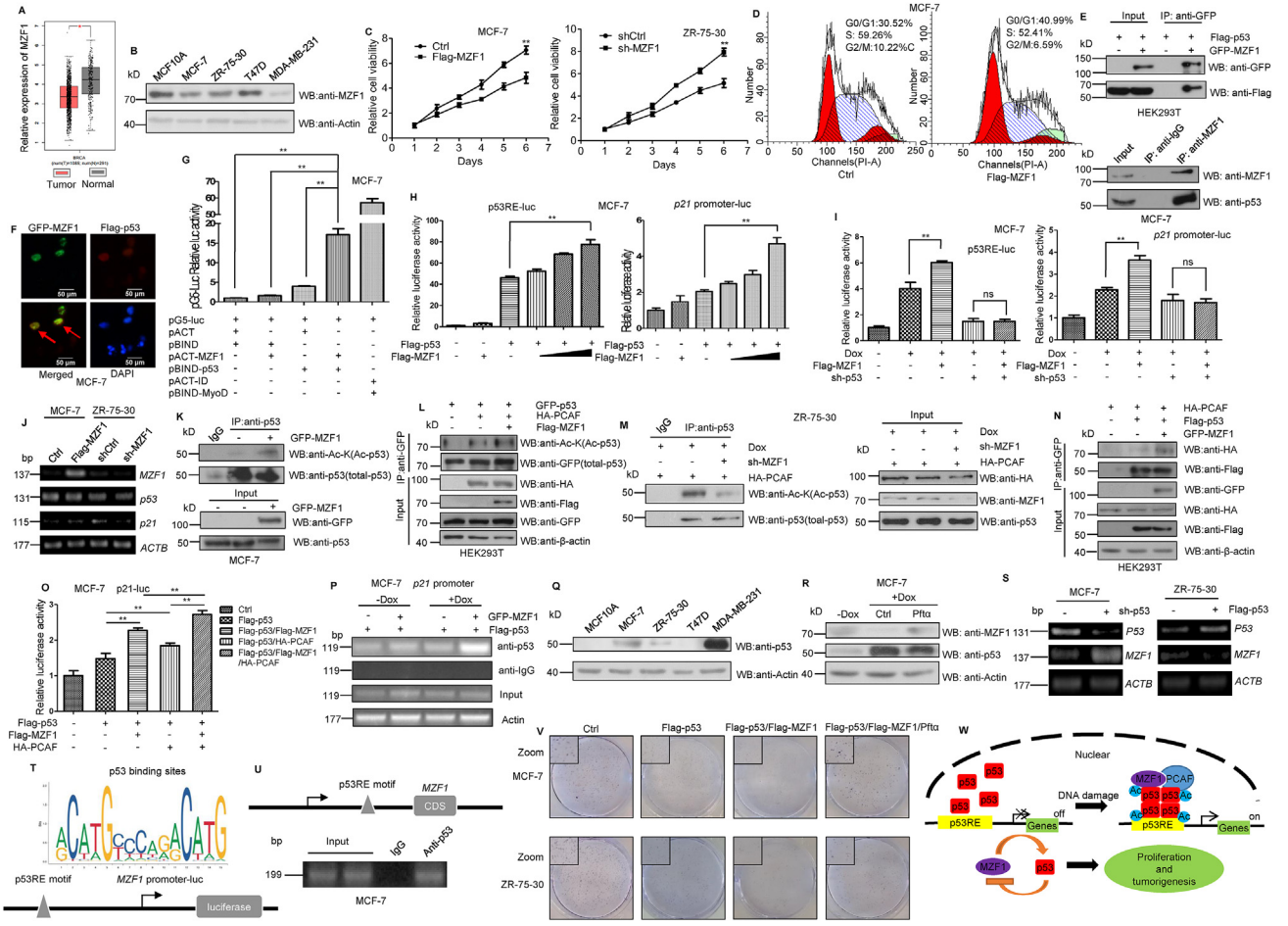

Figure 1 MZF1 suppresses breast cancer cell proliferation by activating the transcriptional activity of p53 and recruiting its acetyltransferase PCAF. **(A)** The box plot compared the relative RNA expression levels of *MZF1* in breast cancer tumors and normal breast tissues using microarray data from public datasets GEPIA (http://gepia.cancer-pku.cn/). **(B)** Western blot analysis of MZF1 expression in MCF10A, MCF-7, ZR-75-30, T47D, and MDA-MB-231 cell lines. **(C)** The growth curves of MCF-7 cells with overexpression of Flag-MZF1 and ZR-75-30 cells with the interference of shMZF1 were measured with CCK8 assay. **(D)** Flow cytometry analysis was performed to test the cell cycle distribution of MCF-7 cells transfected with Flag-MZF1 or the corresponding control vector, respectively. Experiments were repeated at least three times, and the data were presented as means ± standard deviation. $^*P < 0.05$; $^{**}P < 0.01$. **(E)** CoIP assays showed the interaction between exogenous GFP-MZF1 and Flag-p53 in HEK293T cells and between endogenous MZF1 and p53 in MCF-7 cells. **(F)** Immunofluorescence showed that MZF1 (green) and p53 (red) were located in the nucleus (blue) of MCF-7 cells. **(G)** The direct interaction between MZF1 and p53 was identified through mammalian two-hybrid systems. MCF-7 cells were transfected with the indicated vectors for 24 h, and the activity of pSG5 luciferase was measured. **(H)** Luciferase reporter assays showed that the transcription activity of p53 on p53RE-luc and p21 promoter-luc were enhanced by MZF1 in MCF-7 cells. **(I)** Luciferase reporter assays showed that the activation effect of MZF1 on p53RE-luc and p21 promoter-luc was diminished when endogenous p53 was knocked down by shRNA in MCF-7 cells. ns, no significance. **(J)** RT-PCR assays detected the mRNA level of *MZF1*, *p53*, and p53-target genes *p21* with overexpression of Flag-MZF1 or knockdown of endogenous shMZF1 in MCF-7 and ZR-75-30 cells. **(K)** With the treatment of 1 μM deacetylase inhibitors trichostatin A (TSA), the CoIP assay tested the endogenous acetylation level of p53 with or without overexpression of GFP-MZF1 in MCF-7 cells. **(L)** CoIP assay tested the exogenous acetylation level of p53 with overexpression with Flag-MZF1 and HA-PCAF in HEK293T cells. The relative protein level was normalized with β-actin. **(M)** CoIP assay showed that the endogenous acetylation level of p53 with MZF1 knockdown was lower than that in the control group in ZR-75-30 cells. **(N)** CoIP assay showed that MZF1 enhanced the recruitment of PCAF to p53 in HEK293T cells. **(O)** Luciferase reporter assay showed that MZF1 enhanced the activation of PCAF on the transactivation of p53. **(P)** ChIP assay conducted in MCF-7 cells treated with 0.5 μM Dox (1 h) indicated the activation effect of MZF1 on the enrichment of p53 in the *p21* promoter. **(Q)** The expression level of p53 in MCF10A, MCF-7, ZR-75-30, T47D, and MDA-MB-231 cell lines by Western blot. **(R)** Immunoblotting of MZF1 and p53 in MCF-7 cells treated with or without 0.5 μM Dox and 20 μM Pftα (1 h). **(S)** RT-PCR assays tested the mRNA level of MZF1 in MCF-7 and ZR-75-30 cells with knockdown or overexpression of p53. **(T)** A schematic representation of the MZF1 promoter-luc covered 1000 bp DNA fragment containing the p53RE motif was constructed. **(U)** The p53RE site on the MZF1 potential promoter and the ChIP assay conducted in MCF-7 cells detected the enrichment of p53 binding on the p53RE motif in the MZF1 promoter. **(V)** Crystal violet staining assays evaluating the ability of colony formation of MCF-7 and ZR-75-30 cells, with stable expressing control vector, Flag-p53, Flag-p53/MZF1, and Flag-p53/MZF1 (treated 20 μM Pftα). **(W)** The schematic representation of the regulatory relationship between p53 and MZF1 in breast cancer.

Our previous results showed that MZF1 overexpression could not change the mRNA or protein levels of p53 (Fig. 1J; Fig. S3E—H). Moreover, the p53 stability was not significantly changed by MZF1 (Fig. S4A). Hence, we hypothesized that MZF1 might affect the transcriptional activity of p53 through post-translational modification. Phosphorylation and acetylation are the most important modifications that promote the transcriptional activity of p53.[1] However, a previous study indicated that MZF1 did not influence the phosphorylation of p53.[4] Thus, we proposed that MZF1 may regulate p53 acetylation.[5] As expected, the endogenous acetylation level of p53 was significantly increased with overexpression of MZF1 (Fig. 1K; Fig. S4B). In addition, MZF1 could increase histone acetyltransferases (HATs) p300/CBP-associated factor (PCAF)-mediated acetylation of p53, rather than p300 or Tip60 (Fig. 1L; Fig. S4C—E), and *vice versa* (Fig. 1M; Fig. S4G). Moreover, MZF1 could enhance the interaction between p53 and PCAF (Figs. 1N and S4F). To consolidate this possibility, we found that MZF1 enhanced PCAF-mediated activation on p53 transactivation (Fig. 1O; Fig. S4H, I). Besides, the interaction between MZF1 and PCAF was confirmed (Fig. S4J), and the abundance of p53 in the promoter was significantly enhanced with MZF1 overexpression (Fig. 1P).

Interestingly, a lower level of p53 in cells with high expression of MZF1, and *vice versa* (Fig. 1B, Q; Fig. S1B, 5A). Thus, we speculated there might be a negative correlation between the two proteins and MZF1 might be regulated by p53 signaling. As expected, the endogenous MZF1 was decreased in response to Dox treatment. Consistently, the p53 inhibitor Pifithrin-α (Pftα) reversed the inhibitory effect (Fig. 1R; Fig. S5B). To further consolidate this possibility, the *MZF1* mRNA level was largely increased with *p53* knockdown, and *vice versa* (Fig. 1S; Fig. S5C). Similar results were obtained from its protein level (Figs. S5D, E). Moreover, a conserved p53 response element (p53RE) motif was found at +212 to +227 bp region upstream of the coding sequence (CDS) in the potential promoter of *MZF1* (Fig. 1T). A significantly increased luciferase activity with *p53* knockdown was found in contrast to the control, and *vice versa* (Fig. S5F). In addition, the binding of p53 on this region was confirmed (Fig. 1U).

The association between MZF1 and p53 may participate in the p53-mediated proliferation of breast cancer cells. To test this hypothesis, the growth and colony formation number of cells overexpressing p53 was significantly repressed with overexpression of Flag-MZF1 compared with the control group. Reciprocally, treatment of the cells with Pftα could significantly restore the inhibitory effect (Fig. 1V; Fig. S6A—C). Moreover, the viability of cells overexpressing MZF1 or -Δ3 after the cells underwent *p53* knockdown was not affected in contrast to the control group. Thus, the effect of MZF1 on cell viability may depend on p53 (Fig. S6D—G). In clinical data, among 4929 total breast cancer cases, there were 197 breast cancer patients (wild-type p53) with high MZF1 expression levels who had a significantly longer relapse-free survival and overall survival time than the controls (Fig. S6H, I). However, no significance was found between the 130 breast cancer patients (mutant p53) with high MZF1 expression levels and the controls (Fig. S6J, K).

Collectively, MZF1 could inhibit the proliferation of some breast cancer cells by arresting cells at the G0/G1 phase. In addition, we found that MZF1 had a physical interaction with p53 and activated its transactivation activity. Mechanistically, MZF1 enhanced the connection between p53 and its acetyltransferase PCAF, further inducing PCAF-mediated p53 acetylation and promoting the promoter-binding enrichment of p53 in response to DNA damage. Further study revealed that the expression of MZF1 was repressed by activation of p53 signaling and p53 might directly bind to the *MZF1* potential promoter via recognizing the conserved p53RE motif. Functionally, MZF1 enhanced p53 repressive ability in regulating the proliferation of breast cancer cells. Clinically, a high MZF1 expression was associated with a better prognosis in breast cancer patients, especially with wild-type p53. To sum up, our data suggest a cross-regulatory loop between MZF1 and p53 in breast cancer cells (Fig. 1W). They also provide insight into the mechanism of MZF1 and the approach for molecular-targeted therapy involved in breast cancer with p53.

Author contributions

Conceptualization, W.H., and R.P.; methodology, L.B., and L.S.; validation, L.B., S.X., and Z.Y.; formal analysis, L.B., L.S., and X.Z.; investigation, Y.Y., and Z.B.; resources, Z.B. writing—original draft preparation, L.B., and L.S.; writing—review and editing, L.B., and W.H.; visualization, L.S., and R.P.; supervision, W.H., and R.P.; funding acquisition, W.H. All authors have read and agreed to the published version of the manuscript.

Conflict of interests

The authors declare no conflict of interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.gendis.2022.11.005.

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