Collagen prolyl4-hydroxylase promotes breast cancer endocrine resistance via regulation of cellular stress

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Abstract

Endocrine therapy is the first-line therapy for luminal breast cancer patients. About 30%-50% of luminal breast cancer patients with five to ten years of treatment acquire resistance to endocrine therapy. Understanding the mechanisms of endocrine resistance and identifying new therapeutic targets remain urgent priorities. Here, we show that expression of the alpha subunit of Prolyl hydroxylation (P4HA1), an enzyme hydroxylates proline into 4-hydroxyproline at the Y position of the collagen -X-Y-Gly- triplet motif, is increased in endocrine therapy resistant luminal breast cancer cells compared with parental endocrine therapy sensitive cells. In luminal breast cancer patients, increased P4HA1 expression significantly correlates with short relapse-free survival. Knockdown of P4HA1 or P4HA1 small molecular inhibitors treatment restores endocrine-resistant luminal breast cancer cells sensitivity to endocrine treatment by inhibiting cell proliferation. These results indicate that P4HA1 is an important regulator for endocrine resistance of breast cancer. We also show that the inactivation of P4AH1 combined with endocrine treatment dramatically enhances endoplasmic reticulum (ER) stress-related gene expression and reactive oxygen species (ROS) level. These results indicate that P4HA1 is a key player that coordinates the crosstalk between endocrine resistance and cellular stress. These findings reveal P4HA1 as a novel therapeutic target and hold promising potential of developing new therapeutic strategies for luminal breast cancer patients with endocrine resistance.

Results

Figure 1: Expression of P4HA1 is associated with endocrine resistance.

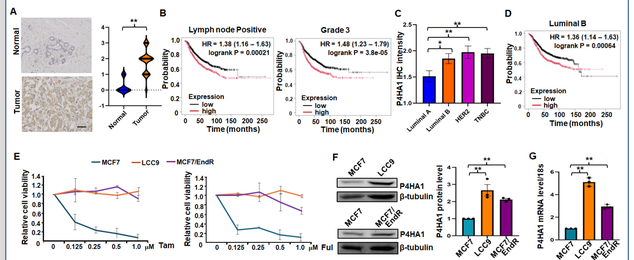


Figure 1. (A) P4HA1 IHC staining in human mammary tumors and adjacent normal tissue. (B) Kaplan Meier analysis of survival in lymph node positive and grade 3 breast cancer. (C) P4HA1 IHC staining in human breast tumors. (D) Kaplan Meier analysis of survival in luminal B breast cancer patients. (E) CCK8 assay detected cell viability of parental MCF7cells, LCC9 cells and MCF7/EndR cells treated with 4OHT/fulvestrant. (F) Western blot analysis P4HA1 protein in parental MCF7 cells, and tamoxifen-resistant LCC9 cells and MCF7/EndR cells (G) qRT-PCR analysis for P4HA1 mRNA in parental MCF7 cells, and tamoxifen-resistant LCC9 cells and MCF7/EndR cells

Figure 2: Knockdown P4HA1 restores endocrine therapy sensitivity.

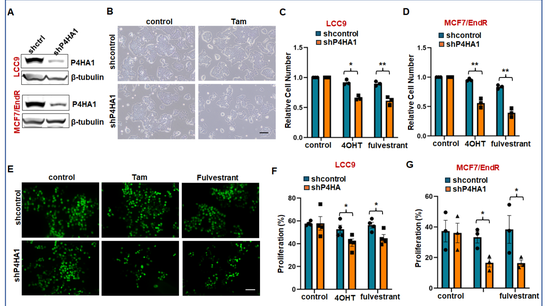


Figure 2. (A) Western blot analysis knockdown efficiency in shP4HA1 LCC9 and MCF7/EndR cells.. (B) Phase images of shcontrol and shP4HA1 LCC9 cells with/without 1 μM fulvestrant, 1 μM 4OHT treatment for 4 days. (C) (D) Quantification relative cell numbers in shcontrol and shP4HA1 LCC9, MCF7/EndR cells with/without 4OHT or fulvestrant. (E) Edu staining analysis cell proliferation in shcontrol and shP4HA1 LCC9 cells with/without fulvestrant, 4OHT treatment. (F) (G) Quantification of cell proliferation in shcontrol and shP4HA1 LCC9 cells with/without fulvestrant, 4OHT treatment. n=3. Results are presented as mean ± SEM, *, P < 0.05; **, P < 0.01.

Figure 3: Inhibition of P4HA1 restores endocrine sensitivity.

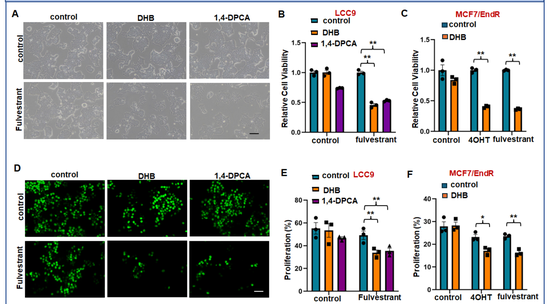


Figure 3. (A) Phase images of LCC9 cells treated with P4HA1 inhibitors DHB, 1,4-DPCA, fulvestrant for 4 days. (B) (C) Quantification relative cell numbers in LCC9 cells and MCF7/EndR cells treated with P4HA1 inhibitors (5 μM 1, 4-DPCA, 20 μM DHB), and/or fulvestrant, or 4OHT for 4 days. (D) Edu staining analysis cell proliferation in LCC9 cells treated P4HA1 inhibitors (1, 4-DPCA, DHB) and fulvestrant for 4 days. (E) (F) Quantification of cell proliferation in LCC9, MCF7/EndR cells treated P4HA1 inhibitors and 4OHT or fulvestrant for 4 days n=3. Results are presented as mean ± SEM, *, P < 0.05; **, P < 0.01.

Figure 4: Inactivation of P4HA1 sensitizes tumor to endocrine therapy agent in vivo.

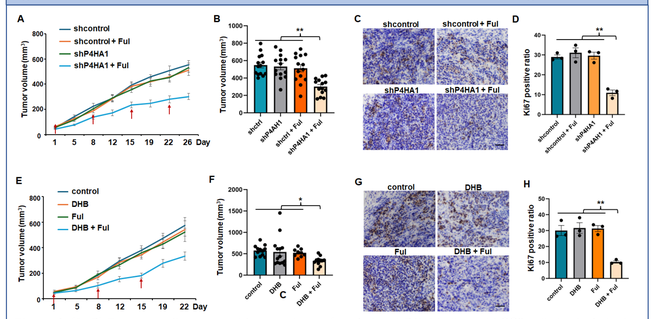


Figure 4. (A, B) Tumor growth curve and tumor volume of control and P4HA1-silenced LCC9 cells implanted mice treated with fulvestrant. Red arrows mark fulvestrant administration. (C, D) IHC images and quantification of Ki67 in control and P4HA1-silenced LCC9 cells tumors excised from SCID mice treated with fulvestrant. (E, F) Tumor growth curve and tumor volume of LCC9 cells implanted mice treated with control, DHB, fulvestrant, DHB combination with fulvestrant. (G, H) IHC images and quantification of Ki67 in tumors excised from SCID mice after four-week DHB and or fulvestrant treatments. Results are presented as mean ± SEM, *, P < 0.05; **, P < 0.01.

Figure 5: Silenced P4HA1 combination with endocrine treatment induces ER stress.

A

GO term	Gene count	p-value	Benjamini
intrinsic apoptotic signaling pathway in response to endoplasmic reticulum stress	5	4.10E-05	2.90E-02
PERK-mediated unfolded protein response	4	4.20E-05	1.50E-02
response to endoplasmic reticulum stress	5	1.00E-03	1.10E-01
type I interferon signaling pathway	5	5.50E-04	7.70E-02

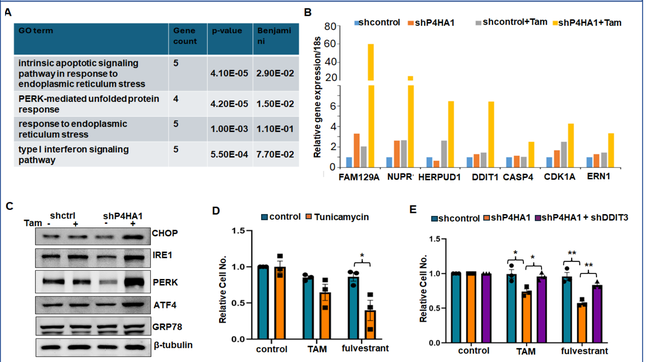


Figure 5. (A) The gene ontology analysis tool analysis the most significantly upregulated pathway in LCC9 shP4HA1 vs shcontrol plus tamoxifen treatment group. (B) Real-time PCR confirm differential gene expression. (C) Western blotting analysis of confirm ER stress pathway protein expression. (D) Cell sensitivity to endocrine treatment combination with ER stress inducer treatment. (E) Silencing CHOP (shDDIT3) restored endocrine resistance suppressed by P4HA1 knockdown. n=3. Results are presented as mean ± SEM, *, P < 0.05; **, P < 0.01.

Figure 6: Inactivation of P4HA1 with endocrine treatment increases ROS level.

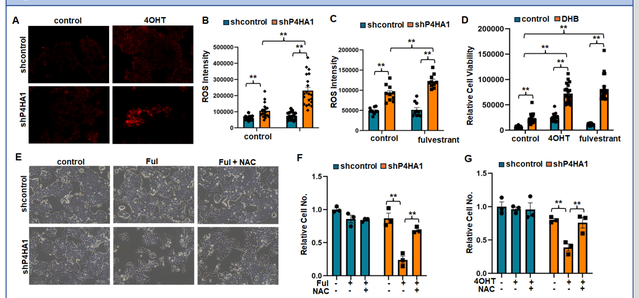


Figure 6. (A) ROS staining images in shcontrol and shP4HA1 LCC9 cells with/without 4OHT treatment for 4 days . (B) (C) Quantification of ROS staining in shcontrol and shP4HA1 LCC9, MCF7/EndR cells with/without 4OHT or fulvestrant treatment. (D) Quantification of ROS staining in LCC9 cells treated P4HA1 inhibitors (DHB) with/without 4OHT or fulvestrant treatment. (E) Phase images of shcontrol and shP4HA1 LCC9 cells treated with fulvestrant with/without ROS inhibitor NAC (1 μM). (F) (G) Quantification of relative cell numbers in shcontrol and shP4HA1 LCC9 cells treated with fulvestrant or 4OHT with/without ROS inhibitor 1 NAC. Results are presented as mean ± SEM, *, P < 0.05; **, P < 0.01.

Conclusions

1. P4HA1 is high expression in luminal B breast cancer and associated with endocrine resistance in luminal breast cancer.
2. Inactivation of P4HA1 restore sensitivity to endocrine treatment in endocrine-resistant luminal breast cancer cells.
3. Target P4HA1 in endocrine-resistant xenograft models resensitizes tumor to endocrine therapy.
4. P4AH1 functional in endocrine sensitivity by regulation cellular stresses including ER-stress and oxidative stress.
5. P4HA1 is a potential therapeutic target for endocrine-resistant luminal breast cancer patients.

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