News Release

Calcineurin-mediated dephosphorylation stabilizes E2F1 protein by suppressing binding of the FBXW7 ubiquitin ligase subunit

Key Points

- We identified FBXW7, a tumor suppressor, as a critical regulator of the degradation of E2F1, a transcription factor that promotes cancer malignancy.
- Calcineurin dephosphorylates E2F1 and stabilizes it by preventing its interaction with FBXW7.
- E2F1 protein levels are regulated by intracellular calcium concentration.

Summary

This study elucidated the molecular mechanism underlying the degradation of E2F1, a transcription factor that drives cancer progression. E2F1 plays a pivotal role in cell cycle regulation and aberrant proliferation of cancer cells, making its degradation an important therapeutic target. We identified FBXW7, a component of the ubiquitin ligase complex, as the key factor responsible for ubiquitinating and promoting proteasomal degradation of E2F1. Phosphorylation of E2F1 at Ser403 facilitates its interaction with FBXW7, leading to degradation. Calcineurin, a calcium-dependent phosphatase, dephosphorylates E2F1 at Ser403, thereby disrupting its interaction with FBXW7 and stabilizing its protein. Moreover, E2F1 levels were modulated by intracellular calcium concentrations. Treatment with calcium channel blockers and calcineurin inhibitors decreases E2F1 expression and inhibits cancer cell proliferation. These findings suggest that targeting calcium signaling and calcineurin may represent promising therapeutic approaches for cancers characterized by elevated E2F1 levels.

Research Background

E2F1 is a key transcription factor that promotes the transition from the G1 to S phase of the cell cycle. Its overexpression has been observed in multiple cancer types and is associated with poor prognosis owing to its role in promoting tumor malignancy and proliferation. Degradation of E2F1 via the ubiquitin-proteasome system is essential for preventing its excessive accumulation, but the precise regulatory mechanisms remain unclear. FBXW7, a tumor suppressor that is mutated or inactivated in various cancers, including colorectal cancer and acute myeloid leukemia, is known to degrade several cell proliferation-associated

proteins. However, its relationship to E2F1 has not yet been established. Calcium signaling, which is critical for cellular homeostasis, plays an important role in various diseases, including cancer and cardiovascular disorders. Calcineurin, a calcium-dependent phosphatase, is central to calcium signaling. Previously, we reported that elevated calcineurin levels in breast cancer activated estrogen receptors, contributing to endocrine therapy resistance and increasing the risk of recurrence. In this study, we identified FBXW7 as a key regulator of E2F1 degradation and revealed the role of calcineurin in modulating this process through dephosphorylation.

Research Results

E2F1 is highly expressed in several cancers and its overexpression correlates with poor prognosis in patients with breast cancer (Figure 1). To clarify the regulatory mechanism of E2F1 degradation, we performed comprehensive screening to identify the ubiquitin ligase responsible for this process (Figure 2). Specifically, we searched for factors that (1) act as ubiquitin ligase complex, (2) are downregulated in cancer, (3) suppress gene expression, and (4) are associated with poor prognosis at low expression levels in breast cancer patients. This led to the identification of FBXW7 as a well-characterized tumor suppressor. E2F1 contains five consensus motifs that can be recognized by FBXW7, and phosphorylation at Ser403 is crucial for its interaction with FBXW7. When phosphorylated, E2F1 is ubiquitinated and degraded by the proteasome, preventing its accumulation in cancer cells. However, calcineurin dephosphorylates E2F1 at Ser403, preventing its interaction with FBXW7 and thus stabilizing E2F1. This highlights the significance of calcium signaling in the regulation of E2F1 stability (Figure 3). Inhibition of calcium signaling with calcium channel blockers reduced intracellular calcium levels, decreased E2F1 expression, and induced cell cycle arrest at G1. Conversely, treatment with the calcium ionophore ionomycin increased E2F1 expression, underscoring the close relationship between intracellular calcium levels and E2F1 stability. Furthermore, in vivo experiments using a tumor xenograft model mouse demonstrated that the calcineurin inhibitor FK506 suppressed tumor growth and reduced E2F1 levels. These findings suggest that FBXW7-mediated degradation of E2F1 is a key regulatory mechanism in cancer and that calcineurin-mediated dephosphorylation plays a crucial role in stabilizing E2F1. These results suggest that calcium channel blockers and calcineurin inhibitors may have therapeutic potential for cancer treatment.

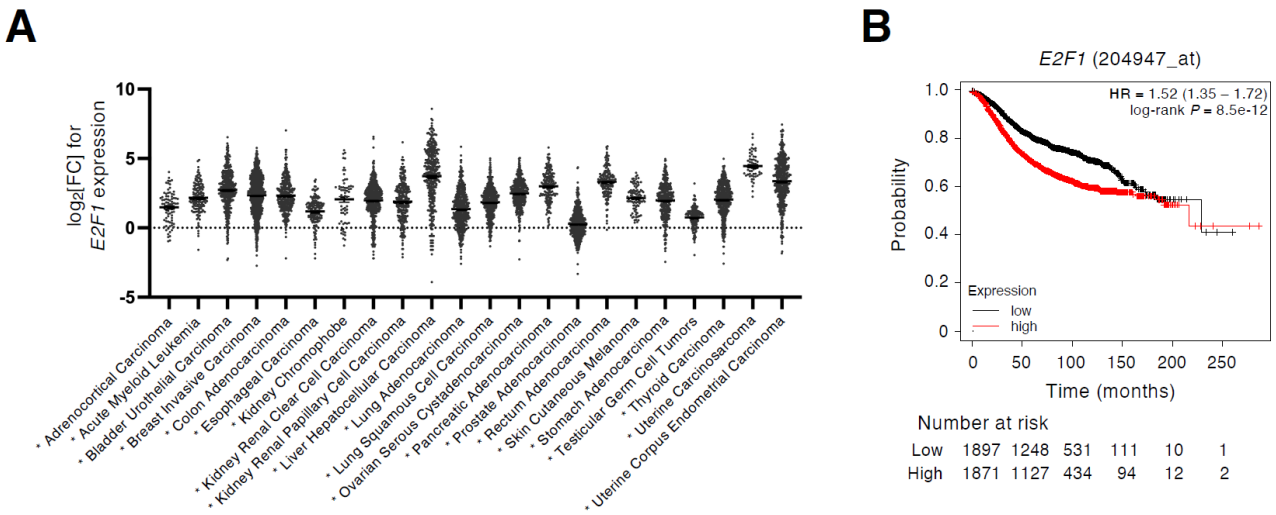

Figure 1: E2F1 expression across multiple cancer types and its correlation with cancer prognosis

A·B: E2F1 mRNA levels are elevated in most cancers and high E2F1 expression is associated with poor prognosis in patients with breast cancer.

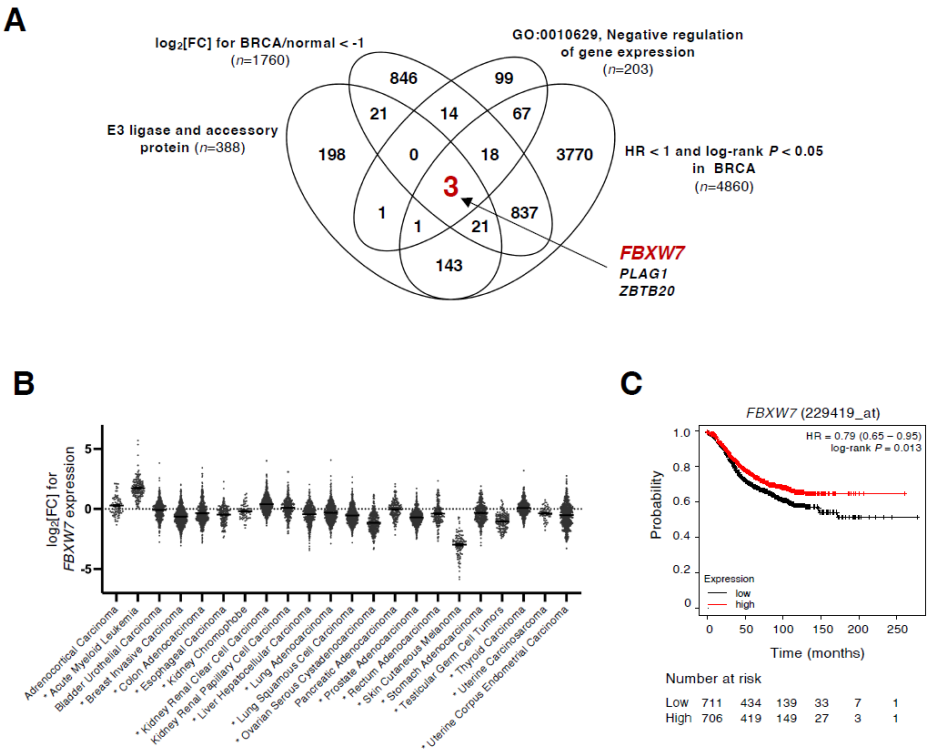

Figure 2: Identification of FBXW7 as a regulator of E2F1 degradation

A. FBXW7 was identified as a ubiquitin ligase for E2F1 using four criteria: (1) its function as a ubiquitin ligase, (2) its downregulation in cancer, (3) its negative regulation of gene expression, and (4) its association with poor prognosis when expressed at low levels.

B·C. FBXW7 mRNA levels are reduced in most cancers, and low FBXW7 expression is correlated with poor prognosis in patients with breast cancer.

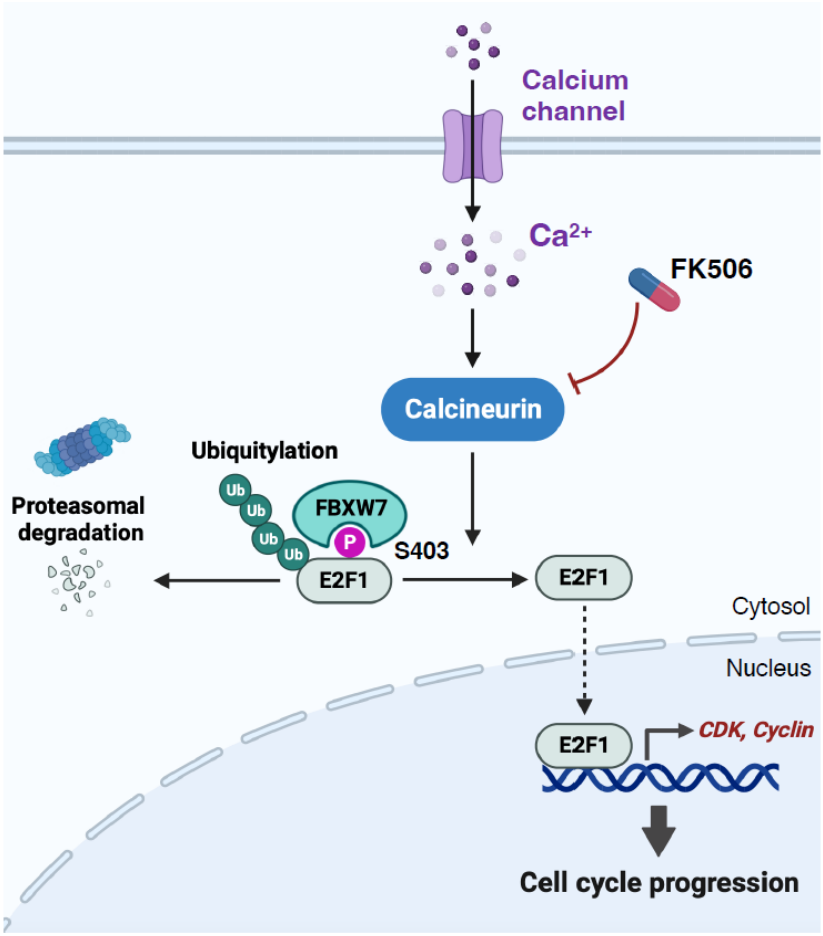

Figure 3: Mechanism of E2F1 regulation by intracellular calcium
Phosphorylation of E2F1 at Ser403 promotes its interaction with FBXW7, leading to its ubiquitination and degradation. Elevated intracellular calcium activates calcineurin, which dephosphorylates E2F1 at Ser403, inhibits its interaction with FBXW7, and stabilizes E2F1. This stabilization promotes the transcription of cell cycle-related genes including CDKs and cyclins.

Research Summary and Future Perspective

This study demonstrates that calcineurin regulates E2F1 stability through dephosphorylation, thereby affecting cancer cell proliferation. These findings suggest that calcineurin inhibitors represent a promising therapeutic approach for cancers driven by E2F1 overexpression. Furthermore, calcineurin inhibition promotes the degradation of other proteins involved in cancer progression. Future studies should explore the efficacy of calcium channels and calcineurin inhibitors across different cancer types and investigate their potential clinical applications.

Publication

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Calcineurin-mediated dephosphorylation stabilizes E2F1 protein by suppressing binding of the FBXW7 ubiquitin ligase subunit

Author:
Yuki Sato[1,a], Makoto Habara[1,a], Shunsuke Hanaki[1], Takahiro Masaki[1], Haruki Tomiyasu[1], Yosei Miki[1], Masashi Sakurai[2], Masahiro Morimoto[2], Daigo Kobayashi[3], Tatsuo Miyamoto[3,4], and Midori Shimada[1,5,*]

Affiliation:
[1]Department of Veterinary Biochemistry, Yamaguchi University
[2]Department of Veterinary Pathology, Yamaguchi University
[3]Department of Molecular and Cellular Physiology, Graduate School of Medicine, Yamaguchi University
[4]Division of Advanced Genome Editing Therapy Research, Research Institute for Cell Design Medical Science, Yamaguchi University
[5]Department of Molecular Biology, Graduate School of Medicine, Nagoya University
[a]Co-first authors
*Correspondence author

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