


COMMENTARY

New relationship of E2F1 and BNIP3 with caveolin-1 in lung cancer-associated fibroblasts

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Abstract

In recent years, studies have found that E2F1, a downstream effector of caveolin-1 (Cav-1), participates in tumor cell metabolic reprogramming. E2F1 modulates mitochondrial fusion and mitophagy. Bioinformatic analysis has identified the E2F1-MFN2 axis as a regulator of mitophagy. Our data establish a new novel paradigm for regulation of the tumor cell metabolic reprogramming pathway by Cav-1 that is operationally linked and mutually dependent on the transcriptional activation of E2F1 and induces mitophagy with BNIP3 in cancer-associated fibroblasts (CAFs).

As a founding member of E2F family of transcription factors, E2F transcription factor 1 (E2F1) participates in regulating cell-cycle progression, cell differentiation, DNA repair, and apoptosis.¹ In recent years, studies have found that E2F1 participates in tumor cell metabolic reprogramming in addition to regulating the cell cycle, and plays an important role in the occurrence and development of tumors.² Depending on the cellular context and environmental conditions, it can function as either an oncogene or a tumor suppressor gene.

Bucha *et al.* found³ that E2F1 modulated mitochondrial fusion and mitophagy, probably through regulation of MFN2. Bioinformatic analysis identified the E2F1-MFN2 axis as a regulator of mitochondrial morphology and mitophagy, which is the most direct evidence to show the relationship between E2F1 and mitophagy. Previous research has showed that BCL2 interacting protein 3 (BNIP3) is a direct transcriptional target of E2F1 that is necessary and sufficient for E2F1-induced mitophagy.⁴ BNIP3 at the outer mitochondrial membrane interacts with processed LC3 at phagophore membranes to promote sequestration of mitochondria within the autophagosome for degradation.⁵

Yurkova *et al.*⁴ provided the first direct evidence that activation of the intrinsic mitochondrial death pathway by E2F1 is mutually dependent on, and obligatorily linked to, the transcriptional activation of BNIP3.

In lung cancer, the normal fibroblasts (NFs) are activated as cancer-associated fibroblasts (CAFs), and act in the realms of the tumor microenvironment (TME) with consequences for tumor growth, formation of stem cell niches, immunosuppression, metastasis and chemoresistance.^{6,7} A number of studies have suggested that CAFs are key in cancer progression. Caveolin-1 (Cav-1) is found predominantly in terminally differentiated cells, such as adipocytes, endothelia and smooth muscle cells, as well as type I pneumocytes.^{8,9} As the first member of the Cav family, Cav-1, a 22 kDa protein of 178 amino acids, has been the most sufficiently investigated in a number of biochemical studies. The downregulation of Cav-1 is a major characteristic of CAFs and existing studies have indicated that CAFs have the ability to prevent cancer cell apoptosis, enhance the proliferation of cancer cells and stimulate tumor angiogenesis.¹⁰ It has also been reported that the downregulation of Cav-1 is one of the mechanisms that mediates the

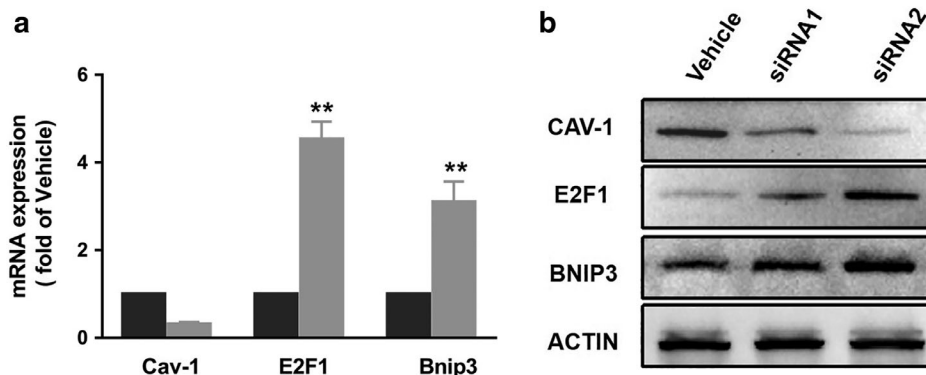


Figure 1 Cav-1 regulates E2F1 and BNIP3 expression in CAFs. (a) CAFs were transduced without (vehicle) or with Cav-1 siRNA, the mRNA expression of Cav-1, E2F1 and BNIP3 in cells were detected after 24 hours treatment, and β -actin was used as an internal control. $**P < 0.01$ versus vehicle (Vehicle ■) (Cav-1 siRNA ▒). (b) CAFs were transduced without (vehicle) or with Cav-1 siRNA, the protein levels of CAV-1, E2F1 and BNIP3 in cells were measured by western blotting after 48 hours treatment, and β -actin was used as a protein-loading control.

transformation of fibroblasts. Recent studies showed the relationship on Cav-1, mitochondria and cancer metabolism indicate the altered metabolism induced by Cav-1 and mitophagy is a major cause of tumor cell metabolic reprogramming.¹⁰

Guruswamy *et al.* reported¹¹ that E2F1, a downstream effector of Cav-1-dependent cell survival signals mediated through Akt activation, is inhibited by a combination of lovastatin and/or celecoxib. However, the research did not clarify the direct correlation between Cav-1 and E2F1, but only reflected E2F1 as a correlation factor downstream of Cav-1. In our research, we demonstrated that CAFs were transduced without (vehicle) or with Cav-1 siRNA (Fig 1a). The mRNA expression of Cav-1, E2F1 and Bnip3 in cells were detected after 24 hours treatment, and β -actin was used as an internal control. The expression of E2F1 and BNIP3 were increased at 24 hours post-transfection in CAFs ($**P < 0.01$ vs. vehicle). A similar significant increase in the expression of E2F1 and BNIP3 at protein level was also observed in Figure 1b.

Indeed, earlier work by our laboratory established that the expression of BNIP3, a mitophagy marker, in CAFs was higher than that in NFs, and the result showed statistical significance. The expression of BNIP3 in CAFs with overexpression of Cav-1 was significantly lower than the expression of BNIP3 in CAFs without overexpression of Cav-1. Therefore, we believe that the expression regulation of Cav-1 has a direct impact and regulation on BNIP3.

To our knowledge, our data provides the first direct evidence that Cav-1 and E2F1 have a very close relationship and that BNIP3 is transcriptionally activated in CAFs myocytes by E2F1 and underlies E2F1-induced mitochondrial defects. Importantly, our finding that E2F1 is activated by Cav-1 is in complete agreement with a report in the literature.¹¹ Although we cannot exclude the possibility

that other factors regulated by Cav-1 may contribute to tumor cell metabolic reprogramming, the notion that knockdown of Cav-1 with siRNA in CAFs causes E2F1 expression to increase directly, is favorable based on our finding that E2F1 engages the BNIP3 promoter coincidentally with the activation of Cav-1 lowly expression. In addition, knockdown of Cav-1 with siRNA in CAFs has a direct impact on E2F1 and then regulation of BNIP3. The findings of the present study provide new mechanistic evidence that Cav-1 is necessary and sufficient for induction of E2F1 activation.

In conclusion, based on the present findings, our data establish a new novel paradigm for regulation of the tumor cell metabolic reprogramming pathway by Cav-1 that is operationally linked and mutually dependent on the transcriptional activation of E2F1.

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Disclosure

The authors declare that they have no competing interests.

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