

Epigenetic suppression of FBXL7 promotes metastasis

Loredana Moro & Michele Pagano

To cite this article: Loredana Moro & Michele Pagano (2020) Epigenetic suppression of FBXL7 promotes metastasis, *Molecular & Cellular Oncology*, 7:6, 1833698, DOI: [10.1080/23723556.2020.1833698](https://doi.org/10.1080/23723556.2020.1833698)

To link to this article: <https://doi.org/10.1080/23723556.2020.1833698>



© 2020 The Author(s). Published with license by Taylor & Francis Group, LLC.



Published online: 22 Oct 2020.



Submit your article to this journal [↗](#)



Article views: 272

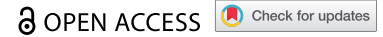


View related articles [↗](#)





View Crossmark data [↗](#)

AUTHOR'S VIEWS



Epigenetic suppression of FBXL7 promotes metastasis

Loredana Moro ^{a,b,c} and Michele Pagano ^{a,b,d}

^aDepartment of Biochemistry and Molecular Pharmacology, New York University Grossman School of Medicine, New York, NY, USA; ^bPerlmutter NYU Cancer Center, New York University Grossman School of Medicine, New York, NY, USA; ^cInstitute of Biomembranes, Bioenergetics and Molecular Biotechnologies, National Research Council, Bari, Italy; ^dHoward Hughes Medical Institute, New York University Grossman School of Medicine, New York, NY, USA

ABSTRACT

Epigenetic reprogramming is emerging as a key mechanism for metastasis development. Our study identified a novel regulatory mechanism whereby promoter methylation-mediated epigenetic silencing of the gene encoding the ubiquitin ligase subunit F-box/LRR-repeat protein 7 (FBXL7) induces accumulation of active c-SRC, which, in turn, activates epithelial-to-mesenchymal transition and supports cancer cell invasion and metastasis.

ARTICLE HISTORY

Received 27 September 2020
Revised 1 October 2020
Accepted 4 October 2020

KEYWORDS

Epigenetics; metastasis; epithelial-to-mesenchymal transition; ubiquitin ligase; c-SRC

Metastatic spread of cancer cells from primary tumors and their subsequent colonization of distant tissues involves multiple sequential steps forming the invasion-metastasis cascade.¹ In contrast with the extensive knowledge of the pathogenic mechanisms leading to tumor development, defining when and how a primary cancer cell becomes metastatic remains a puzzling question. Notably, less than 0.01% of the primary tumor cells intravasating the circulatory system give rise to metastases, suggesting that a relatively small subset of cancer cells in the primary tumor must acquire additional “abilities” to successfully give rise to metastases in distant organs.^{1,2} A large body of evidence supports the idea that gene mutations alone are not “sufficient” to explain metastasis and that epigenetic changes provide the additional “abilities” needed by primary cancer cells to strive and achieve metastatic properties.^{1,3}

Unlike genetic mutations, epigenetic reprogramming provides a long-term regulation that remains reversible, fulfilling the requirement of plasticity of cancer cells during invasion and metastasis. For example, epigenetic changes are involved in the cell plasticity required during epithelial-to-mesenchymal transition (EMT), a complex biological program characterized by loss of the cell epithelial properties and acquisition of mesenchymal, motile traits.⁴ To date, most of our knowledge pertains to epigenetic changes occurring during the initial steps of tumorigenesis but little is known about key epigenetic aberrations leading to cancer progression and metastasis. Epigenetic modifications include DNA methylation of CpG-rich regions (CpG islands), histone modifications, and RNA interference. Notably, methylation of CpG islands in the promoter region of many tumor suppressor genes occurs during cancer progression and is considered a *bona-fide* tumor-driving event.⁵

F-box proteins are substrate receptors for SCF (SKP1, CUL1, F-box protein, RBX1) ubiquitin ligases complexes,

which exert important functions in the regulation of several cancer hallmarks. In Moro *et al.*,⁶ we profiled 15 cancer cohorts of The Cancer Genome Atlas (TCGA) project for gene mutations and promoter methylation of the 69 F-box protein family members. Our analysis confirmed that *F-box and WD repeat domain containing 7 (FBXW7)*, one of the top 20 genes mutated in human cancers, was the most highly mutated gene within the F-box protein family, but we found that other members were rarely mutated. The promoters of many tumor suppressors are hypermethylated in human cancers to stably silence their expression as an alternative mechanism to gene loss or mutations. Thus, we next analyzed the promoter methylation of the 69 human genes encoding F-box proteins. We found that the promoter of the gene encoding F-box/LRR-repeat protein 7 (FBXL7) is frequently hypermethylated in human cancers. We focused our attention on pancreatic cancer, one of the most aggressive cancer types because it forms metastases very early, and on prostate cancer, a slow-progressing cancer. Our study revealed that *FBXL7* promoter hypermethylation was associated with reduction of FBXL7 mRNA and protein levels in pancreatic carcinomas and late-stage prostate cancers. Survival analysis of prostate and pancreatic cancer patients in the TCGA dataset showed that low levels of FBXL7 mRNA correlated with reduced patients' survival. These findings suggest that suppression of FBXL7 expression represents a negative prognostic marker and that epigenetic silencing of *FBXL7* might be one of the key events leading to cancer progression and a metastatic phenotype.

To test the hypothesis that FBXL7 acts as a tumor suppressor, we investigated the oncogenic potential of FBXL7 downregulation in pancreatic and prostatic cancer cells. FBXL7 knockdown significantly increased cell motility and invasion, but not cell proliferation. We then analyzed

potential molecular effectors of FBXL7-mediated suppression of cancer cell invasion. We found that the proto-oncogene c-SRC is an FBXL7-binding partner and that FBXL7 mediates the ubiquitylation and degradation of active c-SRC upon its phosphorylation on Ser104 (Figure 1). C-SRC is a non-receptor tyrosine kinase overexpressed in many solid tumors, mostly at later stages of cancer progression.⁷ Mechanistically, active c-SRC mediates EMT and increases the migratory and invasive ability of cancer cells by phosphorylating a plethora of protein substrates, including focal adhesion kinase (FAK) and β -catenin.^{6,8} Notably, high c-SRC activity in prostate and pancreatic cancers predicts poor prognosis and directly correlates with vascular invasion, presence of lymph node metastases, and reduced patients' survival.⁸⁻¹⁰ We found that FBXL7 and c-SRC protein levels are inversely correlated in prostatic and pancreatic human cancer specimens. When we injected prostate and pancreatic prostate cells in orthotopic mouse models, we observed that FBXL7 knockdown resulted in the formation of distant metastases and increased levels of the EMT markers zinc finger E-box binding homeobox 1 and 2 (ZEB1 and ZEB2) in the primary tumors. We also found that genetic silencing of c-SRC or administration of dasatinib, a Food and Drug Administration (FDA)-approved c-SRC inhibitor, prevented metastases in these model systems, but did not affect the growth of the primary tumor, indicating that inhibition of

c-SRC activity may be clinically relevant in a neoadjuvant setting to prevent cancer dissemination, but not to reduce the growth of the primary tumor.

Since the *FBXL7* promoter is hypermethylated in cancer, we analyzed the effect of decitabine, an FDA-approved DNA-methylase inhibitor, in preventing invasion and metastasis. Decitabine treatment increased FBXL7 protein and mRNA expression, thus confirming that epigenetic silencing is a relevant mechanism controlling FBXL7 expression. Recovery of FBXL7 expression upon decitabine treatment was accompanied by decreased levels of active c-SRC and by inhibition of EMT and invasion. *In vivo*, decitabine treatment reduced the size of the primary tumors both in control and FBXL7 short hairpin RNA (shRNA)-expressing mice, but prevented the formation of distant metastases only in control mice. This finding has important translational implications since it suggests that decitabine is effective in inhibiting metastatic dissemination of pancreatic and prostatic cancer cells in an FBXL7-dependent manner, thus highlighting the potential clinical efficacy of decitabine treatment in preventing metastatic dissemination of prostate and pancreatic cancers that exhibit *FBXL7* promoter hypermethylation.

Collectively, our findings highlight an anti-metastatic role of *FBXL7* whereby epigenetic silencing of *FBXL7* by promoter methylation promotes the metastatic spread of pancreatic cancers and late-stage prostate adenocarcinomas. Since *FBXL7*

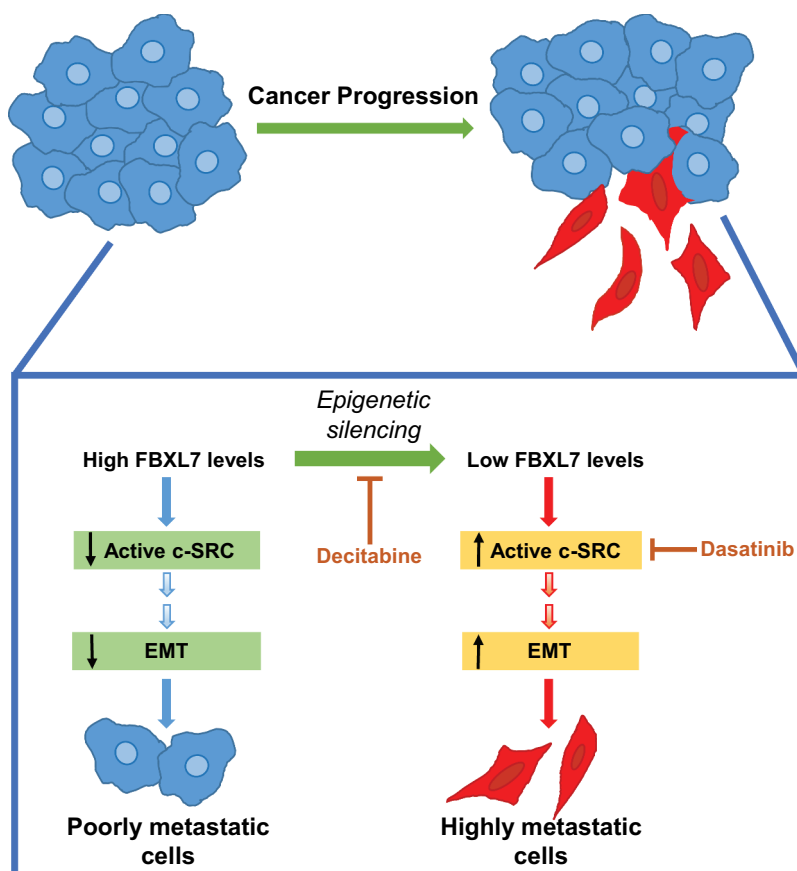


Figure 1. Epigenetic suppression of F-box/LRR-repeat protein 7 (FBXL7) promotes an invasive phenotype in pancreatic and prostatic cancer cells. FBXL7 binds and ubiquitylates active c-SRC leading to its degradation. Epigenetic silencing of *FBXL7* by promoter methylation increases the levels of active c-SRC and activates epithelial-to-mesenchymal transition (EMT), promoting invasion and metastasis. Treatment with the demethylating agent decitabine or inhibition of c-SRC activity with the c-SRC inhibitor dasatinib reverses the metastatic phenotype induced by loss of FBXL7.

promoter methylation occurs in many cancers, our findings may have implications in other tumor types.


Disclosure of potential conflicts of interest

L.M. declares that she has no conflict of interest. M.P. has financial interests in Coho Therapeutics, CullGen Inc., and Kymera Therapeutics. M.P. is on the SAB of CullGen Inc. and Kymera Therapeutics. M.P. is a consultant for Coho Therapeutics, CullGen Inc., Kymera Therapeutics, and SEED Therapeutics.

Funding

This work was supported by grants from the National Institutes of Health to M.P. (GM136250 and CA76584). M.P. is an Investigator with the Howard Hughes Medical Institute.

ORCID

Loredana Moro  <http://orcid.org/0000-0002-5111-3465>
Michele Pagano  <http://orcid.org/0000-0003-3210-2442>

References

- Chatterjee A, Rodger EJ, Eccles MR. Epigenetic drivers of tumourigenesis and cancer metastasis. *Semin Cancer Biol.* 2018;51:149–159. doi:10.1016/j.semcancer.2017.08.004.
- Vanharanta S, Massague J. Origins of metastatic traits. *Cancer Cell.* 2013;24(4):410–421. doi:10.1016/j.ccr.2013.09.007.
- Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA, Kinzler KW. Cancer genome landscapes. *Science.* 2013;339(6127):1546–1558. doi:10.1126/science.1235122.
- Skrypek N, Goossens S, De Smedt E, Vandamme N, Epithelial-to-Mesenchymal Transition: BG. Epigenetic reprogramming driving cellular plasticity. *Trends Genet.* 2017;33(12):943–959. doi:10.1016/j.tig.2017.08.004.
- Pfeifer GP. Defining driver DNA methylation changes in human cancer. *Int J Mol Sci.* 2018;19:4. doi:10.3390/ijms19041166.
- Moro L, Simoneschi D, Kurz E, Arbin AA, Jang S, Guaragnella N, Giannattasio S, Wang W, Chen Y-A, Pires G, et al. Epigenetic silencing of the ubiquitin ligase subunit FBXL7 impairs c-SRC degradation and promotes epithelial-to-mesenchymal transition and metastasis. *Nat Cell Biol.* 2020;22(9):1130–1142. doi:10.1038/s41556-020-0560-6.
- Summy JM, Gallick GE. Src family kinases in tumor progression and metastasis. *Cancer Metastasis Rev.* 2003;22(4):337–358. doi:10.1023/A:1023772912750.
- Patel A, Sabbineni H, Clarke A, Somanath PR. Novel roles of Src in cancer cell epithelial-to-mesenchymal transition, vascular permeability, microinvasion and metastasis. *Life Sci.* 2016;157:52–61. doi:10.1016/j.lfs.2016.05.036.
- Tatarov O, Mitchell TJ, Seywright M, Leung HY, Brunton VG, Edwards J. SRC family kinase activity is up-regulated in hormone-refractory prostate cancer. *Clin Cancer Res.* 2009;15(10):3540–3549. doi:10.1158/1078-0432.CCR-08-1857.
- Lutz MP, Esser IB, Flossmann-Kast BB, Vogelmann R, Lührs H, Friess H, Büchler MW, Adler G. Overexpression and activation of the tyrosine kinase Src in human pancreatic carcinoma. *Biochem Biophys Res Commun.* 1998;243(2):503–508. doi:10.1006/bbrc.1997.8043.