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MYC retards cancer cell migration through suppressing fibronectin expression

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The oncoprotein MYC is a transcription factor that regulates a range of genes involved in various biological processes including cell cycle arrest, cell proliferation, metabolism, protein synthesis and genomic instability. Recently, MYC has been shown playing a role in regulating cancer cell metastasis, while outcomes of this regulation remain controversial. In some breast tumors, MYC accelerates metastasis by elevating miR-9 miRNA expression [1]. However, under certain circumstance, MYC activation may have an opposite function in suppressing cell motility and impairing cancer invasion [2–4]. Intriguingly, through direct suppressing expressions of αv and β3 integrin subunits, MYC impedes breast cancer cell metastasis [3]. Moreover, MYC blocks JNK signaling and consequently causes reduced tumor cell migration [5]. Therefore, the role of MYC in cell migration is largely unknown and needs further investigation.

Cell migration and invasion are controlled by extracellular and intracellular signals, and rely on the interaction between cell and the ligand molecules in the extracellular matrix (ECM) [6–8]. Fibronectin (FN) is a ubiquitously expressed extracellular matrix protein that binds to membrane-spanning receptor proteins called integrins [9]. Fibronectin plays an essential role in cell adhesion, migration, and wound healing [10,11]. Fibronectin affects the formation of proper substratum for migration and growth of cells during the development and organization of granulation tissue, as well as remodeling and resynthesis of the connective tissue matrix [12]. Furthermore, Fibronectin has been implicated in carcinoma development [13]. In lung carcinoma, Fibronectin expression is upregulated especially in non-small cell lung carcinoma.

We report a new mechanism for MYC-mediated suppression of cancer cell migration and invasiveness. Originally, to evaluate the role of MYC in cell migration, we performed both wound-healing and transwell migration assays. Knockdown of MYC in human bone osteosarcoma epithelial U2OS cells strongly enhanced directional cell migration toward a "wound" in a cell monolayer (Fig. 1a). Similar results were obtained in human prostate cancer DU145 cells and PC3 cells (Fig. S1a, b online). Conversely, enforced expression of MYC significantly reduced cell migration (Fig. 1b). These findings were also confirmed by transwell experiments using a permeable filter. Overexpression of MYC markedly decreased cell migration through a permeable filter (Fig. 1c). Together, these findings suggest that MYC may suppress cancer cell migration.

Cell motility is driven by dynamic changes in cell polarization, actin remodeling and small GTPase proteins recruitment. To investigate the mechanism by which MYC-medicated cell migration, we tested whether MYC regulates expressions of genes encoding proteins functionally involved in cell motility. Interestingly, among these proteins including the small GTPase proteins (Cdc42, Rac1 and RhoA), ECM proteins (Fibronectin, Vimentin and Vitronectin), MMP family (MMP2 and MMP9) and growth factors (TGFβ and EGF), mRNA levels of Fibronectin and its receptors (integrin α5 and β1) were remarkably decreased in U2OS cells when MYC was overexpressed (Fig. 1d). Conversely, silencing of MYC augmented the expressions of Fibronectin, Integrin α5 and β1 (Fig. 1e, S2a online). Correlating with these mRNA data, depletion of MYC led to increased protein levels of Fibronectin (Fig. 1f). By contrast, enforced expression of MYC resulted in a decrease in the protein expression of Fibronectin (Fig. 1g). To generalize these findings, we examined the effect of MYC on Fibronectin in DU145 cells and PC3 cells. Similarly, siRNA-mediated silencing of MYC in these cells resulted in a significant increase in both mRNA and protein levels of Fibronectin (Fig. S2b, c online). Conversely, when MYC was overexpressed, Fibronectin mRNA level was strongly reduced (Fig. S2d online). Collectively, these data indicate that MYC has a role in inhibiting Fibronectin expression.

By analyzing the human *Fibronectin* gene sequence for potential MYC response element (E-boxes), we identified a potential response element (GCGCGC) in the first intron of *Fibronectin* gene (Fig. 1h). To investigate the binding of MYC to this response element (RE), we performed chromatin immunoprecipitation (ChIP) assays and found that, in DU145 cells, endogenous MYC bound to the response element region of *Fibronectin* gene (Fig. 1i). To determine whether this response element confers MYC-dependent transcriptional activity, we cloned DNA fragments containing the response element into the promoter region of a firefly luciferase reporter plasmid. Compare to vector control, introduction of MYC induced luciferase expression from the plasmid containing the response element (Fig. 1j). Thus, these findings demonstrate that Fibronectin is a transcriptional target for MYC.

We next wanted to know whether repression of Fibronectin expression contributes to MYC-mediated cell

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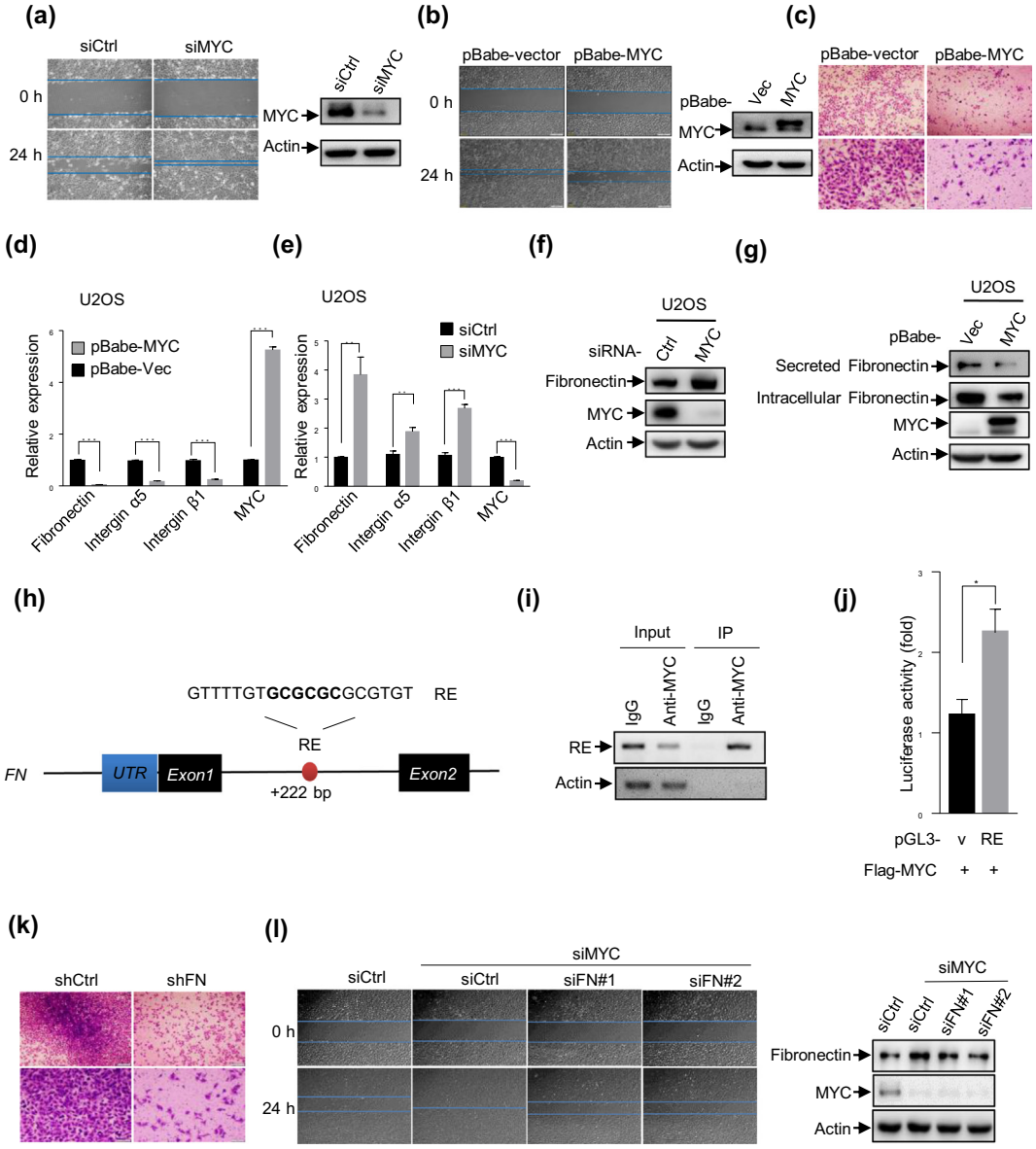

Fig. 1. MYC inhibits cell migration through transcriptionally downregulating Fibronectin expression. (a) Effects of MYC depletion on migration of cultured U2OS cells were examined by wound-healing assay. The protein expression was determined by using western blot. U2OS cells stably overexpressing of MYC or vector control were examined by wound-healing (b) and Matrigel migration assays (c) respectively. Protein expression was analyzed. All results are representative of three independent experiments. (d) Quantitative RT-PCR (qRT-PCR) analysis of total mRNA from U2OS cells stably expressing MYC or vector control. (e) U2OS cells were transfected with MYC siRNA or control siRNA. 48 h later, total mRNA was extracted and analyzed by qRT-PCR. Cell lysates from U2OS cells treated with MYC siRNA or control siRNA (f), or stably overexpressing MYC or vector control (g) were analyzed by western blotting. (h) Schematic representation of human Fibronectin genomic structure. Shown are the exon/intron organization, consensus MYC protein response element (RE) within the first intron. (i) DU145 cells were analyzed by ChIP assay with IgG or MYC antibodies. Data represent two independent experiments. (j) Luciferase reporter construct containing MYC RE were transfected into 293T cells together with Flag-MYC plasmid. Renilla vector pRL-CMV was used as transfection internal control. Relative levels of luciferase are shown. Data are means ± s.d. (n = 3 independent experiments). (k) U2OS cells stably expressing a control shRNA (shCtrl) or shRNA against *Fibronectin* gene (shFN) were examined by Matrigel migration assay. (l) U2OS cell were transfected with control siRNA, MYC siRNA and/or Fibronectin siRNA as indicated. Wound-healing assay was examined. Protein expression was determined by western blot analysis.

migration suppression. Interestingly, stably knocked down Fibronectin in U2OS cells markedly reduced cell migration (Fig. S3a online). Similar result was observed in PC3 cells (Fig. S3b online). Consistently, silencing Fibronectin with short interfering RNA (siRNA) impeded cell migration (Fig. S3c online). Moreover, when depletion of Fibronectin resulted in decreased numbers of cells migrated through a permeable filter (Fig. 1k). Importantly, knocking down the expression of MYC significantly increased the cell migration in U2OS cell, whereas this effect was abrogated when Fibronectin was depleted (Fig. 1l). Similar results were observed in PC3 cells. Fibronectin depletion near completely suppressed MYC silencing-induced PC3 cell migration (Fig. S3d, e online). Together, these observations suggest that MYC has a role in suppressing cell migration, which is largely dependent on Fibronectin.

We demonstrate that MYC retards cell migration through Fibronectin, an extracellular matrix protein. Mechanistically, MYC can directly bind to *Fibronectin* gene and repress the expression of Fibronectin. In support of this notion, silencing of MYC in different cancer cell lines leads to enhanced cell migration, and Fibronectin depletion significantly attenuated the inhibitory effect of MYC on cell migration. Previously, it has been reported that MYC decreases breast cancer cell motility and invasion by transcriptional suppressing of integrin subunits [3]. Additionally, MYC suppresses cell migration in *Drosophila* and human lung adenocarcinoma cell lines

in a JNK-dependent manner via directly upregulating *puc* transcription [5]. These all together suggest a possibility that MYC depletion in human cancers might accelerate metastasis and invasion, inadvertently revealing a potential pitfall of therapeutic targeting MYC. Nevertheless, our results suggest that Fibronectin may be an essential mediator in cancer metastasis and could be a therapeutic target for tumors lacking of MYC.

Conflict of interest

These authors declare that they have no conflict of interest.

Acknowledgments

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scib.2019.04.027.

References

[1] Ma L, Young J, Prabhala H, et al. miR-9, a MYC/MYCN-activated microRNA, regulates E-cadherin and cancer metastasis. Nat Cell Biol 2010;12:247–56.
[2] Waikel RL, Kawachi Y, Waikel PA, et al. Deregulated expression of c-Myc depletes epidermal stem cells. Nat Genet 2001;28:165–8.
[3] Liu H, Radisky DC, Yang D, et al. MYC suppresses cancer metastasis by direct transcriptional silencing of alphav and beta3 integrin subunits. Nat Cell Biol 2012;14:567–74.
[4] Cappellen D, Schlange T, Bauer M, et al. Novel c-MYC target genes mediate differential effects on cell proliferation and migration. EMBO Rep 2007;8:70–6.
[5] Ma X, Huang J, Tian Y, et al. Myc suppresses tumor invasion and cell migration by inhibiting JNK signaling. Oncogene 2017;36:3159–67.
[6] Guo W, Giancotti FG. Integrin signalling during tumour progression. Nat Rev Mol Cell Biol 2004;5:816–26.
[7] Hood JD, Cheresh DA. Role of integrins in cell invasion and migration. Nat Rev Cancer 2002;2:91–100.
[8] Li T, Kon N, Jiang L, et al. Tumor suppression in the absence of p53-mediated cell-cycle arrest, apoptosis, and senescence. Cell 2012;149:1269–83.
[9] Pankov R, Yamada KM. Fibronectin at a glance. J Cell Sci 2002;115:3861–3.
[10] Grinnell F. Fibronectin and wound healing. J Cell Biochem 1984;26:107–16.
[11] Valenick LV, Hsia HC, Schwarzbauer JE. Fibronectin fragmentation promotes alpha4beta1 integrin-mediated contraction of a fibrin-fibronectin provisional matrix. Exp Cell Res 2005;309:48–55.
[12] Grinnell F, Billingham RE, Burgess L. Distribution of fibronectin during wound healing *in vivo*. J Invest Dermatol 1981;76:181–9.
[13] Han S, Khuri FR, Roman J. Fibronectin stimulates non-small cell lung carcinoma cell growth through activation of Akt/mammalian target of rapamycin/S6 kinase and inactivation of LKB1/AMP-activated protein kinase signal pathways. Cancer Res 2006;66:315–23.

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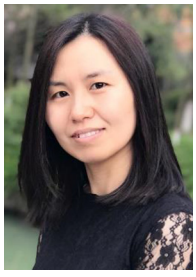

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