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MYC Suppresses Cancer Metastasis by Direct Transcriptional Silencing of αv and β3 Integrin Subunits

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Abstract

Over-expression of MYC transforms cells in culture, elicits malignant tumors in experimental animals and is found in many human tumors. We now report the paradoxical finding that this powerful oncogene can also act as a suppressor of cell motility, invasiveness and metastasis. Overexpression of *MYC* stimulated proliferation of breast cancer cells both in culture and in vivo as expected, but inhibited motility and invasiveness in culture, and lung and liver metastases in xenografted tumors. We show further that MYC represses transcription of both subunits of αvβ3 integrin, and that exogenous expression of β3 integrin in human breast cancer cells that do not express this integrin rescues invasiveness and migration when MYC is downregulated. These data uncover an unexpected function of MYC, provide an explanation for the hitherto puzzling literature on the relationship between MYC and metastasis and reveal a variable that should influence the development of therapeutics that target MYC.

The proto-oncogene *MYC* encodes an exceptionally pleiotropic transcription factor (MYC) that participates in the control of a wide variety of genes[1-3]. Included among these genes are functions vital to regulation of the cell cycle, cell growth, apoptosis, cell adhesion, and genomic stability[3-5]. Over-expression of MYC transforms cells in culture[6], elicits tumors in experimental animals[7], and is found in as many as 50% of all human cancers and 25% of human breast cancers[8-13]. Paradoxically, such overexpression is on occasion dissociated

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AUTHOR CONTRIBUTIONS

H.L., D.C.R., D.Y., E.S.R., M.J.B. and J.M.B designed the research; H.L., D.C.R., R.X., E.S.R., and D.Y. performed research; H.L., D.Y., D.C.R., E.S.R., M.J.B and J.M.B. analyzed data; H.L., D.C.R., M.J.B. and J.M.B. wrote the paper.

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from the propensity to invade and metastasize[10,13-18]. These observations raise the possibility that MYC may inhibit cellular properties such as motility and invasion that are essential to metastasis. Consistent with this possibility, overexpression of *MYC* in mouse skin causes a severe impairment in wound healing and in keratinocyte migration, whereas deletion of *MYC* causes increased migration of keratinocytes[19,20].

Cellular invasion and migration are governed by extracellular and intracellular signals, and depend on the interaction of the cell with ligand molecules in the extracellular matrix (ECM)[21-23]. The principal ECM receptors are the integrins, and altered expression of integrins is associated with tumor growth and metastasis[24,25]. Overexpression of *MYC* was found to inhibit the spreading and adhesion of primary keratinocytes, which exhibited decreased expression of α6, β1 and β4 integrins in response to *MYC*[26]. When *MYC* was overexpressed in cells from neuroblastomas and sarcomas, the levels of α3 and β1 integrins were down-modulated[27,28], whereas genetic ablation of *MYC* in hematopoietic stem cells stimulated the expression of α2, α5, and β1 integrins, and other ECM proteins[29]. In addition, MYC binds to the E-box sequence of the promoters of the α3 and α7 integrin genes and silences their transcription in mouse myoblast and human sarcoma cells[27,30].

We report here that elevated expression of MYC reduced the motility and invasiveness of breast cancer cells in vitro, their capacity for local invasion, and their ability to seed distant metastases. Concomitantly, MYC overexpression inhibited the formation of stress fibers and focal adhesions. These effects of MYC could be attributed to the decreased expression of αv and β3 integrins, mediated by repression of transcription from the corresponding genes. Our results provide an explanation for the dissociation between overexpression of *MYC* and metastasis, point to αv and β3 integrins as crucial elements in the metastasis of malignant cells, and uncover a variable that may be important in the development of therapeutics that target MYC.

RESULTS

MYC inhibits invasion and metastasis

We explored the role of MYC in metastasis by manipulating expression of the gene in four established cell lines of human breast cancer, two of which express little MYC (MDA-MB-231 and BD549), and two of which express MYC at high levels (MCF-7 and T47D) (Fig. 1a), and in human retinal pigment epithelium (RPE) cells, a cell line that has been used previously for investigating the effects of *MYC* on induction of proliferation and apoptosis.

Exogenous overexpression of human *MYC* in MDA-MB-231, BT549, and RPE cells significantly increased proliferation assessed by BrdU labeling (Fig. 1b) without enhancing basal levels of cell death (Fig. 1c). However, overexpression of *MYC* significantly inhibited migration of these cells (Fig. 1d) and reduced invasiveness of MDA-MB-231 and BT549 cells (Fig. 1e). Overexpression of *MYC* in MDA-MB-231 and BT549 cells also inhibited the invasive growth pattern of these cells when cultured in three-dimensional, laminin-rich gels (lrECM) such as Matrigel (Fig. 1f). When injected subcutaneously, *MYC*-expressing MDA-MB-231 cells produced tumors that showed substantially reduced local invasion (Fig. 1g), even though the expression of MYC led to significantly larger tumors (Fig. 1h). These

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results showed that overexpression of *MYC* simultaneously promotes cell proliferation while inhibiting invasion, both in culture and *in vivo*.

Invasion and metastasis are both characteristics of cancer progression. We found that MDA-MB-231 cells overexpressing MYC showed significantly reduced incidence of lung metastases following injection into the tail vein (Fig. 2a,b), although occasional *MYC*-expressing cells that did metastasize resulted in larger tumors (Fig. 2c). To assess tumor growth and metastasis from the orthotopic site, MDA-MB-231-vector and MDA-MB-231-MYC cells were transduced with luciferase-expressing virus (Fig. 2d) and injected into the inguinal mammary gland. We found that the *MYC*-expressing tumors, as expected, grew more rapidly at the orthotopic site (Fig. 2e-g). However, isolation and quantification of luminescence of lungs (Fig. 2h,i) and livers (Fig. 2m,n) revealed decreased metastatic burden at 6 weeks following injection, though the difference was not statistically significant. Metastatic burden is a combination of both number of metastases and the size of the metastatic tumors; we found that expression of MYC significantly reduced the number of metastases but led to increased size of individual metastases in both lung (Fig. 2j-l) and liver (Fig. 2o-q). We conclude that overexpression of MYC can stimulate cell proliferation producing larger tumors that have reduced invasiveness and metastatic potential. These findings are consistent with recent indications that dissemination of tumor cells may occur early during tumor progression and is not necessarily linked to primary tumor size[31].

MYC modulates cell interaction with the extracellular matrix

Motility is a dynamic process associated with major changes in cellular phenotype including spreading, actin polymerization, formation of actin-rich protrusions at the leading edge of migrating cells, and creation and dissolution of focal adhesions during cell translocation[32,33]. We found that exogenous overexpression of *MYC* in both MDA-MB-231 and RPE cells (Fig. 3a,b) reduced cell spreading, decreased stress fiber and focal adhesion formation, and increased cortical actin (Fig. 3c,d). Since formation of focal adhesions requires the interaction of cell surface integrins with their ligands in the extracellular matrix (ECM), we hypothesized that the effect of overexpression of *MYC* on focal adhesion might reflect a defect in the interaction of cells with specific ECM ligands. To test this hypothesis, we assessed cell attachment to purified human ECM ligands and found that overexpression of *MYC* in MDA-MB-231 or RPE cells reduced their attachment to vitronectin (Fig. 3e,f). Such defects could in turn impair cellular motility. To explore this possibility, we measured chemotaxis towards purified vitronectin in Boyden chamber assays, and found that overexpression of *MYC* in MDA-MB-231 and RPE cells decreased migration of both cell types (Fig. 3g,h).

MYC inhibits expression of αv and β3 integrins

To explore the mechanism by which *MYC* impedes invasion and motility, we measured the expression of subunits of αvβ3 integrin, which is a well-known cell surface receptor for vitronectin[34]. Overexpression of *MYC* in RPE, MDA-MB-231 and BT549 cells was accompanied by substantial reductions in both integrin subunits in all three cell lines, and less consistent reductions of α5, β1 and β5 subunits (Fig. 4a). Conversely, when the high levels of endogenous *MYC* in MCF7 and T47D cells were knocked down by siRNA, the

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levels of αv, α5, β1 and β5 integrin subunits rose (Fig. 4a and data not shown). Irrespective of the level of MYC expression, β3 integrin could not be detected by either RT-PCR or western blots in MCF7 cells[35] (Fig. 4a, and data not shown), nor in T47D cells (see below, Fig. 6c). Hence, both cell lines appear to be intrinsically deficient in β3.

Overexpression of *MYC* in MDA-MB-231 cells caused a reduction of RNA for both αv and β3 (Fig. 4b,c). A canonical E-box binding site for MYC is located upstream of the transcription initiation sites for both αv (CACATG) and β3 (CACGTG) integrins in human DNA (Fig. 4d). Quantitative chromatin immunoprecipitation assays (ChIP) showed that MYC bound directly to the E-box region of both these genes in MDA-MB-231 cells (Fig. 4e,f) and in RPE cells (not shown), but not to non-specific sequences in the same domain. Although MYC was originally viewed as a transcriptional activator, it can serve also as a transcriptional repressor[36]. Our results show that overexpression of *MYC* represses transcription of integrin genes involved in migration and metastasis.

Inhibition of invasion and metastasis by MYC depends upon αv and β3 integrin modulation

We found that the invasive and migratory phenotype of MDA-MB-231 and RPE cells was dependent on αvβ3 integrin (Fig. 5a,b, and data not shown) as was the invasive growth of MDA-MB-231 cells in lrECM (Fig. 5c), and that defects in cell invasion and migration caused by overexpression of *MYC* could be partially or completely rescued by expressing αv and β3 integrins in MDA-MB-231 (Fig. 5d), RPE (Fig. 5e), or BT549 cells (Fig. 5f). Invasive growth of MDA-MB-231 in 3D lrECM and cell spreading, focal adhesion and stress fiber formation in RPE cells were rescued as well (Fig. 5g). To evaluate whether exogenous overexpression of αv and β3 integrins was sufficient to reconstitute metastatic capability in cells also overexpressing MYC, we generated MYC-expressing MDA-MB-231 cells that also expressed integrin αv, and integrin β3 using exogenous promoters unaffected by MYC (Fig. 5h). When these cells were implanted orthotopically, ectopic expression of integrins αv and β3 substantially increased lung metastasis (Fig. 5i,j). These results indicate that suppression of integrin αvβ3 expression is the key mechanism by which MYC inhibits breast cancer cell metastasis.

We noticed that depletion of either αv or β3 integrin resulted in a parallel reduction of the other (Figure 5a,b); conversely, exogenous expression of β3 integrin increased the amount of αv integrin (see below, Fig. 6a,b, and Supplementary Figs. 1 and 2). Moreover, exogenous expression of either integrin at least partially rescued the phenotype suppressed by *MYC* (Fig. 5d-g). These findings could be explained by mass action, wherein an increase in either integrin alone can augment the formation of heterodimers and stabilize both components. We explored this possibility by co-immunoprecipitation to detect the formation of heterodimers. We found that transfection of αv and β3, either individually or together, substantially increased the amount of heterodimers in extracts of MDA-MB-231 cells (Supplementary Fig. 1). The relative amounts of heterodimer correlated with the extent to which exogenous expression of the integrins rescued a defect in invasion caused by *MYC* (compare Fig. 5d with Supplementary Fig. 1). We conclude that the defects in motility and invasion elicited by overexpression of *MYC* are due to reduced expression of the αv and β3 integrin subunits, which in turn reduces formation of the heterodimer.

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MYC prevents β3 integrin-induced cell invasion

We also explored the effect of depleting the high level of MYC in MCF-7 cells with RNAi (Fig. 6a). Depletion of MYC significantly reduced cellular proliferation as expected (data not shown), whereas it increased cell adhesion to vitronectin and fibronectin (Fig. 6b), as well as cell spreading, focal adhesion and stress fiber formation (Fig. 6g). However, knockdown of MYC in these cells neither increased cell motility (data not shown) nor invasiveness (Fig. 6j), implicating the absence of β3 integrin in MCF7 cells and the consequent inability to form αvβ3 heterodimers. Accordingly, exogenous expression of β3 integrin in MCF-7 cells (Fig. 6c,i) increased invasiveness (Fig. 6d,j) as well as cell spreading (Fig. 6h); similar effects were seen with exogenous expression of β3 integrin in T47D cells (Fig. 6e,f and data not shown).

MCF7 cells grow in a cuboidal morphology with tight cell-cell junctions that restrict individual cell motility. In order to isolate the inhibitory effect of MYC on cell motility, we simultaneously knocked down E-cadherin and MYC, and expressed exogenous β3 integrin in MCF7 cells (Fig. 6i). This combination greatly increased invasion (Fig. 6j). We conclude that when cell-cell interactions are abrogated, inhibition of αv and β3-integrin expression is the primary barrier to cell invasion in MCF7 and possibly other human breast cancer cells that overexpress *MYC*.

DISCUSSION

MYC has been implicated extensively in tumor growth and cell transformation through studies in cultured cells[6,37], targeted expression in mice[38-40] and retrospective analyses of *MYC* expression in human tumors[8]. *MYC* is highly pleiotropic and plays multiple biological roles in driving tumorigenesis. Examples include the ability to initiate tumorigenesis in mice[7], destabilization of the genome[4] and a requirement for maintenance of established tumors[41]. To this list we add here a previously unrecognized effect of *MYC*: ability to inhibit metastasis through inhibition of transcription of an integrin involved in metastasis.

The possibility that *MYC* could act to inhibit metastasis could have been surmised by the observations that overexpression of the gene is sometimes dissociated from the tendency of tumors to metastasize[13,15,18]. Why this may be the case, however, had not been explored. In the case of human breast cancer, distal metastases may express *MYC* at the level of cognate normal cells, even though the gene is over-expressed in the primary tumor[13]. Similarly, mammary carcinoma and other tumors induced by *MYC* in mice frequently fail to metastasize. Examples include murine mammary cancer elicited by *MYC* under the control of the mouse mammary tumor virus (MMTV) promoter[15,17] and aggressive breast tumors elicited by retroviral transduction of both *MET* and *MYC* into normal breast epithelial cells[18].

Here we explored the impact of *MYC* on metastasis by using cell lines derived from carcinomas of the human breast. Two of these lines express low levels of *MYC* and display a metastatic phenotype, whereas the other two overexpress the gene but do not metastasize. Irrespective of the reason for this variation, the differences in MYC expression allowed reciprocal studies on induction or repression of the metastatic phenotype by manipulation of

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MYC expression and its downstream targets. Clearly human breast cancers that overexpress *MYC* may still metastasize if other factors override its function. In fact our *in vivo* assays demonstrated that expression of MYC will support increased growth of those few metastatic cells that escape the inhibitory function of MYC by means of other mutations or by changes in gene expression (Fig. 2m,r).

Given this complexity, we sought direct experimental demonstration that *MYC* can indeed inhibit metastasis. Using MDA-MB-231, the human cell line that expresses only low levels of *MYC* and is used extensively for metastatic studies, we confirmed their highly metastatic potential, by both tail vein injection (Fig. 2c,d) and orthotopic implantation (Fig. 2i-r) in mouse models. However, when exogenous *MYC* was over-expressed in these cells, their ability to metastasize was greatly reduced. Further, the behavior of breast cancer cell lines was remarkably malleable when the level of *MYC* was modulated: it was possible to reduce the metastatic properties by over-expressing *MYC*, or to augment those properties by reducing *MYC* levels. At the same time, *MYC* could increase proliferation even in overtly malignant cells (Fig. 1b). We reported previously an apparent dichotomy between stimulation of proliferation and inhibition of other malignant properties of breast cancer cells also for the oncogene, *AKT*1 (protein kinase B1)[42-44].

Our finding that *MYC* overexpression leads to inhibition of cell motility and invasiveness through direct downmodulation of αv and β3 integrin subunits supports reports that integrins with these subunits are involved in cellular motility, invasiveness, adhesion, and transmigration through endothelium, and are associated with metastasis[22,24,35,45,46]. There are reports also that *MYC* may be involved in controlling the expression of integrin genes using cells from different tissues[26,28,29]. However, none of these reports has linked the effect of *MYC* on integrin expression to cellular components of metastasis, and none has implicated the αvβ3 heterodimer directly in the cellular changes elicited by *MYC*. Our results demonstrate that overexpression of *MYC* represses transcription from the promoters of the αv and β3 integrin genes, which in turn reduces properties that are essential for metastasis. These data, however, do not mean that MYC affects invasion and metastasis only through regulation of integrins αv and β3, nor that MYC is the sole and only regulator of integrin αvβ3 function in invasion and metastasis. The fact that MYC siRNA increases invasion when MCF7 cells are forced to have high levels of integrins αv and β3 (Fig. 6j) suggests that there may be mechanism(s) other than integrin αvβ3 by which MYC can inhibit invasion and metastasis. Nevertheless, since exogenous expression of these integrins is sufficient to bypass the repressive effect of MYC in invasion (Fig. 6d,f,j) and metastasis (Fig. 5h,j), it is apparent that a primary effect of MYC on invasion in breast cancer cells *is* mediated through its inhibition of integrins αv and β3.

Tumor progression often culminates in metastatic disease indicating that there must be selection for antidotes to the *MYC* inhibition of the metastatic phenotype. One obvious possibility would be selection against overexpression of *MYC* during the course of tumor progression. Indeed, the two breast cancer cell lines used in the present study that express relatively low levels of *MYC* both originated from metastatic tumors: MDA-MB-231 from pleural effusion and BT-549 from an invasive ductal tumor in regional lymph nodes. Other possibilities include loss of β3 integrin functions, as in the case of MCF-7 and T47D cell

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lines. Constitutive signaling from active Ras or overexpression of Bcl-XL can also override the inhibition of metastasis by *MYC* (unpublished results of H.L and D.Y). These complexities might account for the fact that in some experimental circumstances, *MYC* may appear to favor metastasis[47-49]. The findings reported here prompt a cautionary note about therapeutic strategies involving *MYC*. The frequency with which *MYC* is over-expressed, and the variety of tumors in which that over-expression occurs, have made *MYC* a seemingly advantageous therapeutic target[50]. Our findings raise the possibility that inhibition of *MYC* in human tumors might at times be contraindicated since its suppression may indeed promote metastasis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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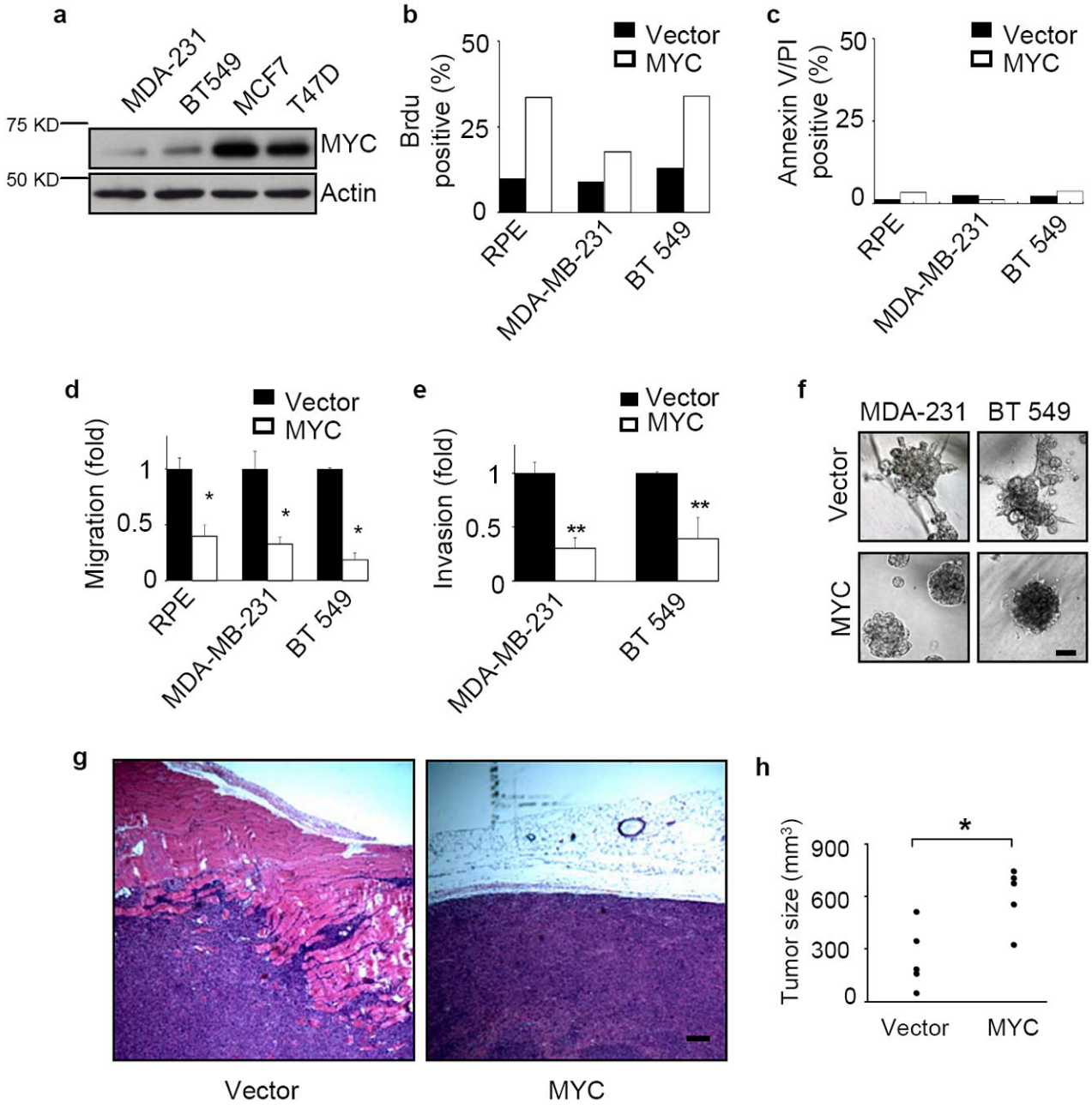

Figure 1.

Elevated *MYC* expression impedes the invasiveness of human breast cancer cells. (**a**) MYC expression levels in four breast cancer cell lines. The endogenous MYC expression levels of MDA-MB-231, BT549, MCF7, and T47D cells were measured by western blots. (**b-c**) Ectopic overexpression of *MYC* increases proliferation but does not affect apoptosis. Cells were labeled with Brdu on tissue culture plastic for 30 minutes at 37°C (**b**, n=2) or incubated with annexin V and propidium iodide (PI) for 5 minutes at room temperature (**c**, n=2). Both positive and total nuclei were counted and the results are expressed as mean±SD, * $p<0.05$. (**d**) MYC overexpression inhibits cell migration. Migration of MDA-MB-231, BT549 and

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RPE cells was measured in Boyden-chamber assays (n=3 for each experiment). The cells were transfected with either vector control or a *MYC* construct. (**e**) The invasiveness of breast cancer cells is inhibited by *MYC* overexpression. Cell invasion through Matrigel-coated transwells was measured for MDA-MB-231 (n=3) and BT549 (n=3) cells stably transduced with vector control or exogenous *MYC*. (**f**) High level of *MYC* expression abrogates the invasive phenotype of breast cancer cells grown in 3D Matrigel. Results are shown for day 6. Scale bars: 50 μm. (**g-h**) *MYC* overexpression enhances tumor growth but reduces tumor invasion into nearby tissues. MDA-MB-231 cells stably expressing vector or exogenous *MYC* were inoculated subcutaneously into nude mice. The tumors were collected 4 weeks after injection, sectioned and stained with hematoxilin and eosin (H&E, **g**) and assessed for size (**h**, n=5 for each group, $p<0.01$). Scale bar: 200 μm. Results are expressed as mean± SD. * $p<0.05$; ** $p<0.01$.

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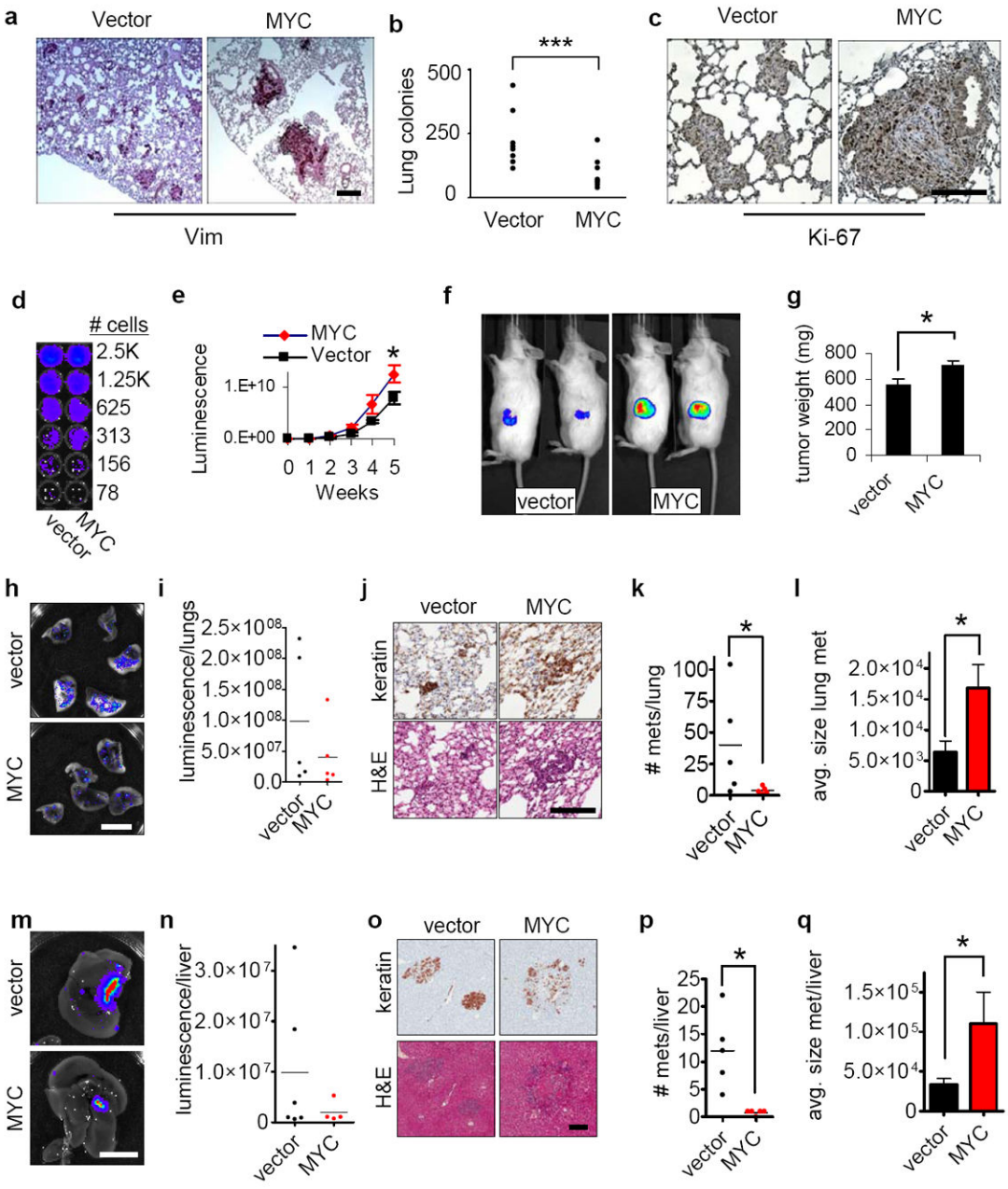

Figure 2.

Elevated *MYC* expression inhibits metastasis of human breast cancer cells. (**a-c**) Over-expression of *MYC* significantly decreases lung metastases of breast cancer cells. MDA-MB-231 cells stably expressing vector or ectopic *MYC* were injected into nude mice through the tail vein and lung metastases were assessed 6 weeks after injection. Sections of the lungs were stained with antibody against human vimentin (**a**) and quantification revealed that MYC significantly reduced lung metastasis (**b**, n=9, p<0.0049). Assessment of Ki-67 (**c**) revealed increased proliferation in the MYC-expressing cells. Scale bars: 200 μm. (**d**) Luminescence detection shows comparable luminescence per cell for both vector and MYC over-expressing cells. (**e**) *MYC*-expressing cells show more rapid growth at the primary

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tumor site, assessed by quantitative in vivo imaging (n=5 for each group). (**f**) Sample images from *in vivo* imaging of primary tumors for vector and *MYC* (week 6). (**g**) Increased size of primary tumors from *MYC*-expressing cells (week 6; n=5 for each group). (**h-i**) Decreased metastatic burden in lungs of mice implanted with *MYC*-expressing cells, assessed by luminescence (week 6; **h**, sample images, scale bar 1 cm; **i**, quantification of lung luminescence; n=5 for each group; differences between cells expressing vector alone and those expressing *MYC* were not statistically significant). (**j**) Images of lung metastases, stained for human cytokeratins (top), and with H&E (bottom). scale bar=200 μm. (**k-l**) Quantification of number (**k**) and size (**l**) of lung metastases indicates that *MYC* cells form much fewer metastases but grow to larger size (n=5 for each group). (**m-n**) Decreased metastatic burden in livers of mice implanted with MYC-expressing cells (week 6; **m**, sample images, scale bar 1 cm; **n**, quantification of liver luminescence; differences between conditions were not statistically significant; n=6 for vector, n=5 for *MYC*). (**o**) Images of liver metastases, stained for human cytokeratins (top), and with H&E (bottom). Scale bar=200 μm. (**q-r**) Quantification of number (**p**) and size (**q**) of liver metastases indicates that MYC-expressing cells form fewer metastases that grow to larger size (n=5 for each group). Results are expressed as mean± SEM. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$.

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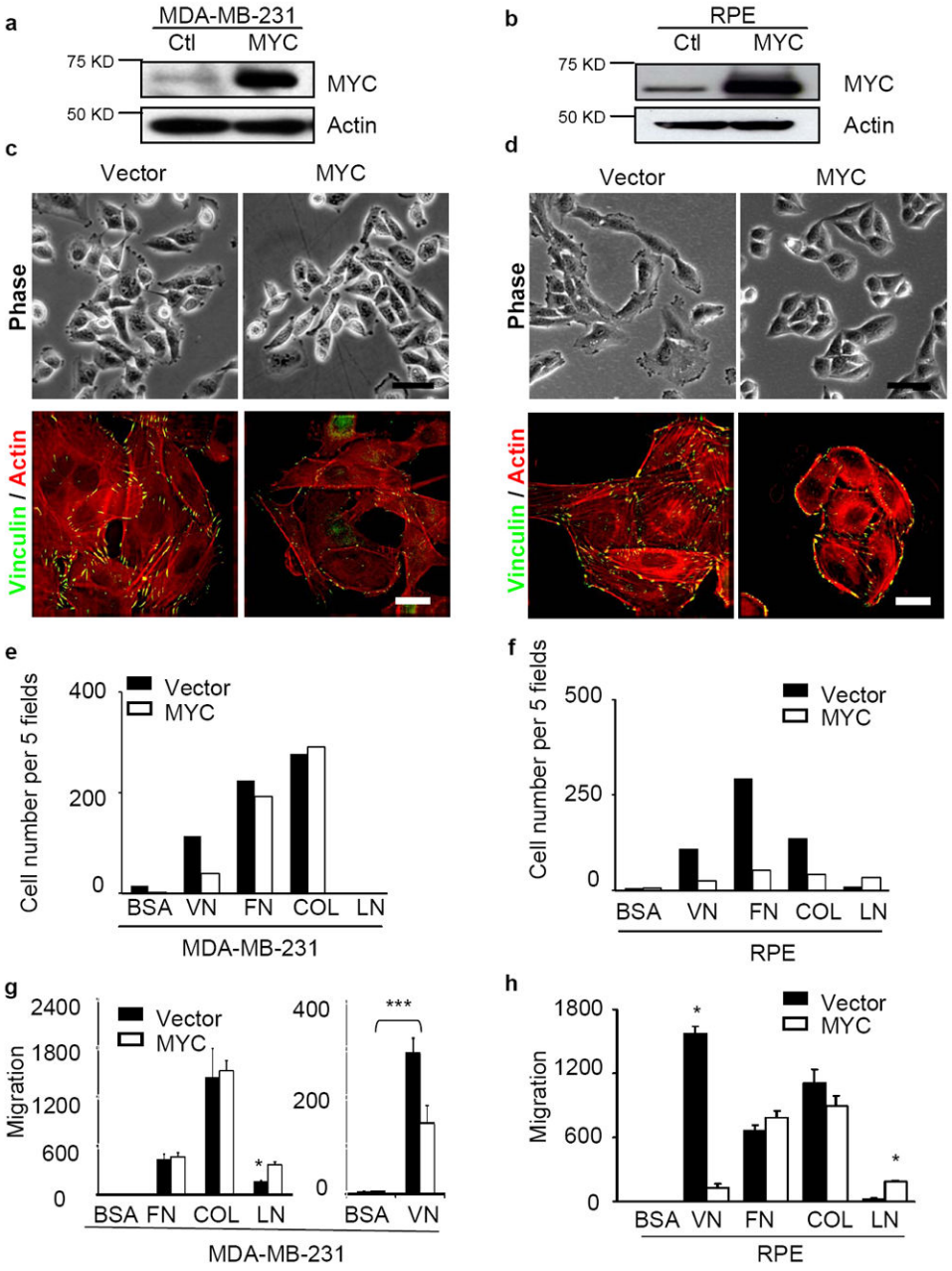

Figure 3.

MYC modulates cell shape, actin cytoskeleton, focal adhesion formation, adhesion to and migration towards ECM. (**a-b**) Overexpression of ectopic *MYC* in MDA-MB-231 (**a**) and RPE (**b**) cells assessed by western blot. (**c-d**) *MYC* overexpression inhibits cell spreading, stress fiber and focal adhesion formation. MDA-MB-231 (**c**) and RPE (**d**) cells expressing vector or ectopic *MYC* were cultured for 24 hr, and then stained with anti-vinculin antibody (green) and Texas-Red-conjugated Phalloidin (red). Scale bars: 20 μm for phase contrast and 5 μm for immunofluorescence staining. (**e-f**) *MYC* overexpression reduces cell adhesion to ECM. MDA-MB-231 (**e**) and RPE (**f**) cells in serum-free medium were plated into 96-well plates coated with purified matrix proteins (VN: vitronectin, FN: fibronectin, Col I: collagen

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I, LN: laminin; n=2). After 45 minutes, adhered cells were counted in five fields. (**g**-**h**) Increased *MYC* expression blocks cell migration towards vitronectin in a Boyden-chamber assay of MDA-MB-231 (**g**) and RPE (**h**) cells (n=3). The results of cell adhesion assay are expressed as mean± SD, *, $p<0.05$.

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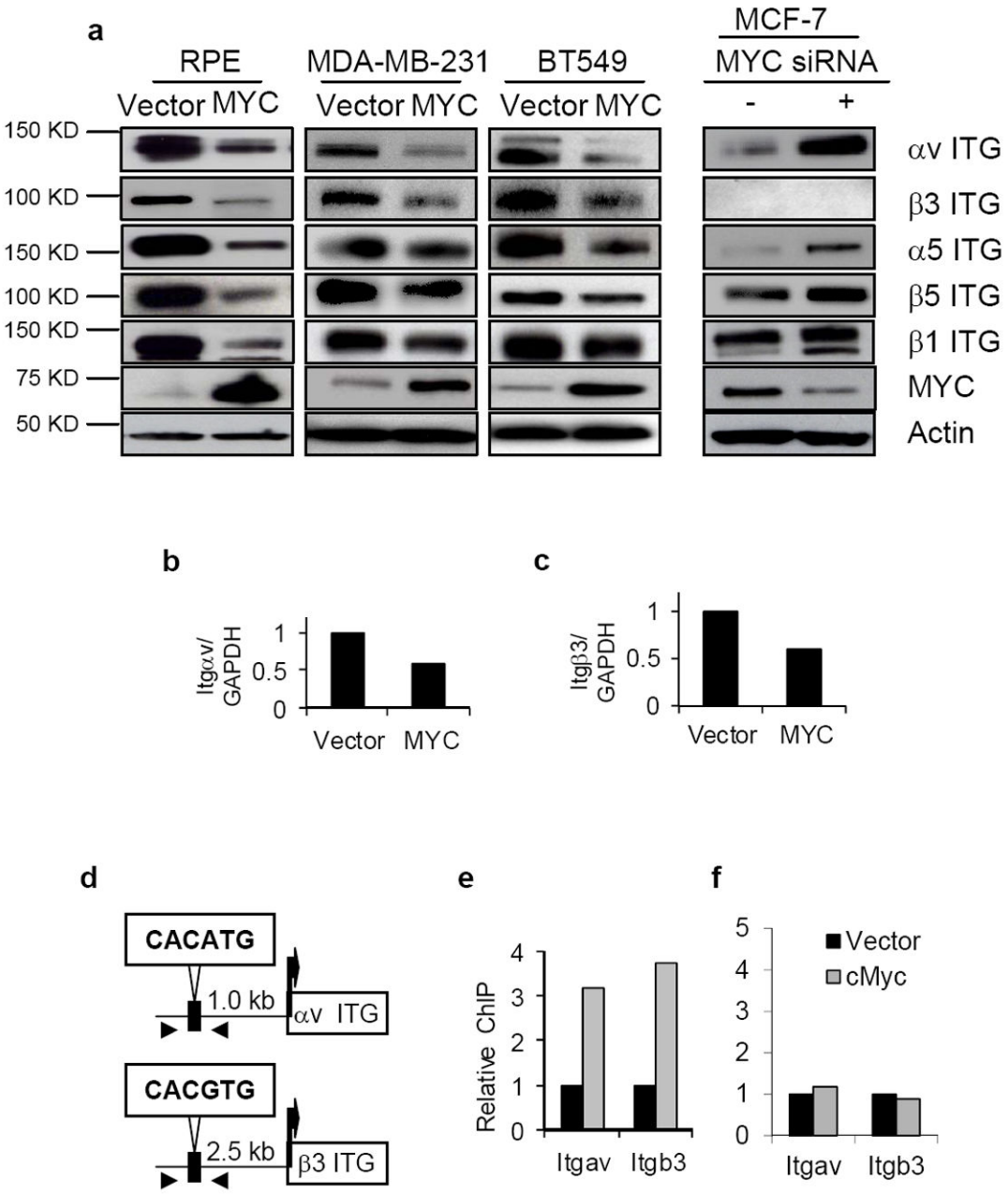

Figure 4.

MYC down-regulates the expression of αv and β3 integrin genes through binding to their proximal promoters. (**a**) *MYC* modulates the abundance of αv and β3 integrins. Total lysates of the indicated cells were fractionated and immunoblotted with the antibodies against the integrins as shown. (**b-c**) Quantitative PCR assessment of integrin αv (Itgav; **b**) and β3 (Itgb3; **c**) expression in response to *MYC* expression in MDA-MB-231 cells (n=2 for each experiment). (**d**) Schematic illustration of the E-box motif upstream of αv and β3 integrin genes; arrowheads locate the designed primers for ChIP assay. (**e-f**) MYC binding to the proximal promoter of αv and β3 integrin genes as determined by ChIP assay. Cross-linked nuclear extracts of MDA-MB-231 transduced with vector only (black columns) or *MYC*

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(grey columns) were immunoprecipitated by either anti-MYC antibody or a control IgG. The regions of the MYC binding sites (**e**) or nonspecific sites (**f**) upstream of αv or β3 integrin genes were quantified and normalized to IgG pulldown (n=2 for each experiment). Data are expressed as mean± SEM. *, $p<0.05$; **, $p<0.01$; ***, $p<0.005$.

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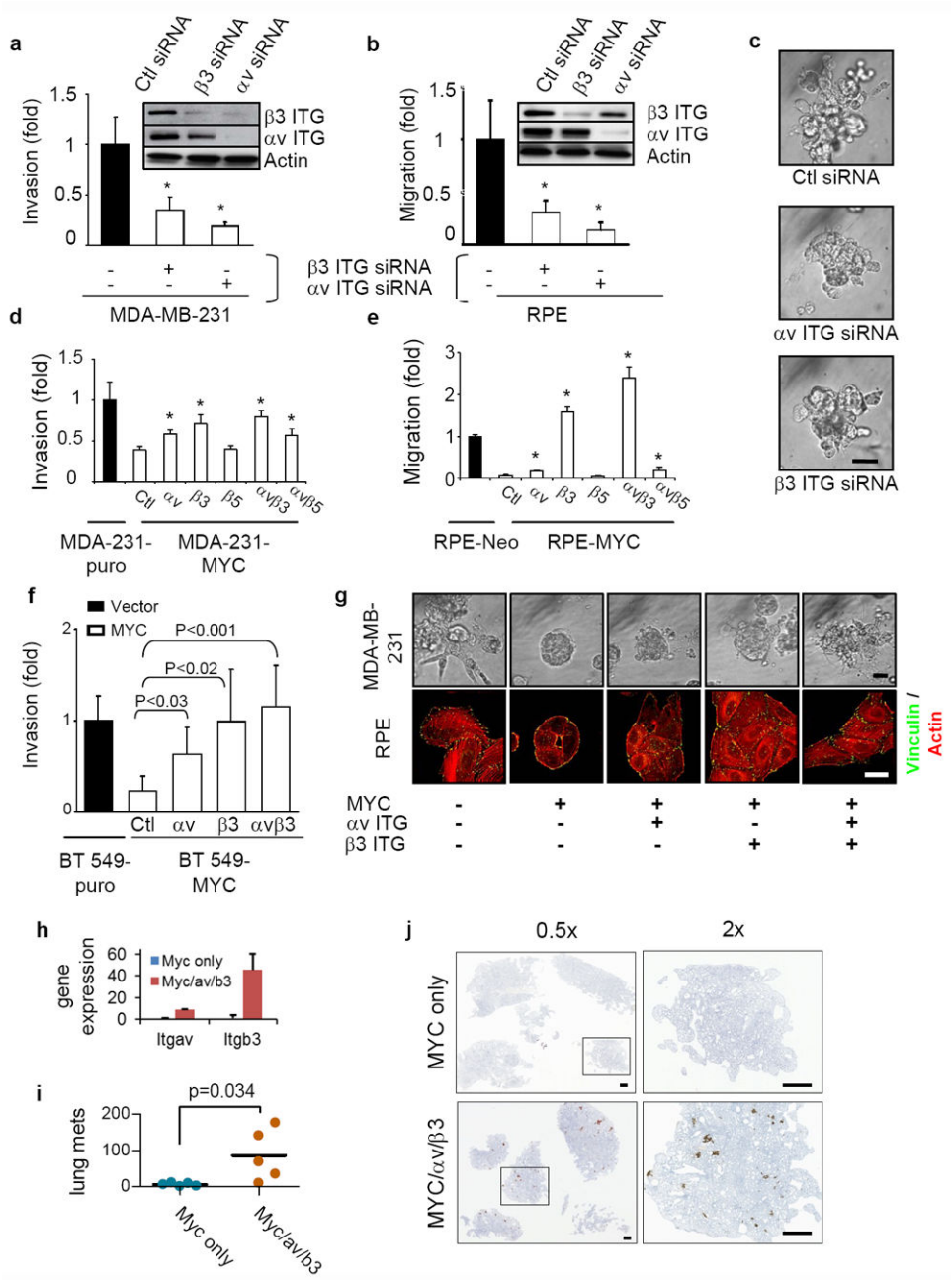

Figure 5.

MYC affects breast cancer cell invasiveness by suppressing integrin αv and β3 subunits. (**a-b**) Knockdown of αv or β3 integrin inhibited invasion (**a**) and migration (**b**) in a Boyden-chamber assay, n=3. Knockdown of the integrins by siRNA was confirmed by western blots. (**c**) Knockdown of αv or β3 integrin inhibits the invasiveness of MDA-MB-231 cells grown in a 3D Matrigel assay for 6 days, n=3. Scale bar: 50μm. (**d-e**) αv and β3 integrin rescues the compromised migration (**d**) and invasiveness-(**e**) elicited by high *MYC* expression, n=3 for each. MDA-MB-231 and RPE cells overexpressing *MYC* were transiently transfected with vector, αv, β3, or β5 integrin constructs. Cell invasiveness and migration were assessed by

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Boyden chamber assay. (**f**) Inhibition of cancer cell invasiveness by *MYC* over-expression can be rescued by exogenous expression of αv and β3 integrin subunits in BT549 cells (n=3). (**g**) Expression of exogenous αv and β3 integrin partially rescued actin cytoskeleton, focal adhesion formation of RPE cells grown on 2D tissue culture plastic dishes, and the compromised invasiveness of MDA-MB-231 cells in a 3D Matrigel assay. RPE or MDA-MB-231 cells, stably expressing the indicated constructs, were plated on cell culture dishes for 24 hours or in 3D Matrigel for 6 days. RPE cells were then stained with anti-vinculin antibody (green) and Texas-Red-conjugated Phalloidin (red). Images of 3D Matrigel culture were obtained by phase contrast microscopy. Scale bars: 50 μm for phase contrast and 5 μm for immunofluorescence staining. (**h**) Quantitation of Itgav and Itgb3 transcripts by quantitative PCR in MDA/MYC and MDA/MYC/αv/β3 cells, n=3. (**i**) Quantitation of increased lung metastases in mice orthotopically implanted with MDA/MYC/αvb3 cells as compared to MDA/MYC cells, n=5 for each. (**j**) Images of lungs of mice orthotopically implanted with MDA/MYC/αvβ3 or MDA/MYC cells. Results are expressed as mean± SD. *, $p<0.05$.

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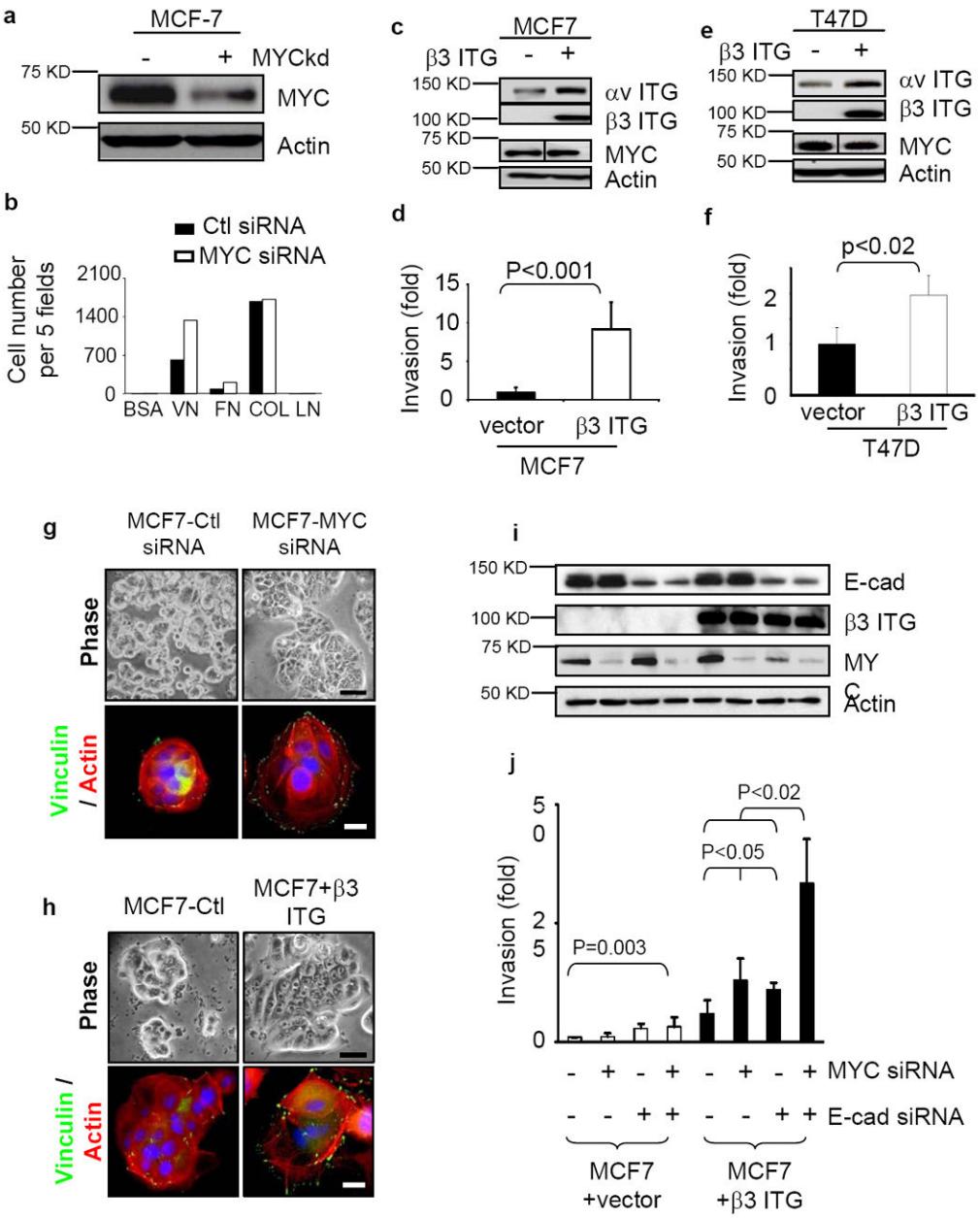

Figure 6.

MYC and E-cadherin can prevent β3 integrin-induced invasion. (**a**) Suppression of *MYC* expression by siRNA in MCF7 cells assessed by western blot. (**b**) Suppression of *MYC* by siRNA augments cell adhesion to vitronectin and fibronectin. The cells in serum-free medium were plated into 96-well plates coated with purified matrix proteins (VN: vitronectin, FN: fibronectin, Col I: collagen I, LN: laminin). After 45 minutes, adhered cells were counted in five fields, n=2. (**c-f**) Ectopic expression of β3 integrin in MCF-7 (**c**) and T47D (**e**) cells, assessed by Western blot, enhanced invasiveness (**d,f**; n=3), as assessed by Boyden chamber assay. (**g-h**) Cell spreading, stress fiber and focal adhesion formation are enhanced in MCF7 cells when MYC is depleted by siRNA (**g**) or when β3 integrin is exogenously expressed (**h**). Changes in cell shape, actin cytoskeleton and focal adhesion

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formation were demonstrated by phase contrast (top) and staining with anti-vinculin (green) and Texas red Phalloidin (bottom). Scale bar: 20 μm for phase contrast and 5 μm for immunofluorescence staining. (**i-j**) Decreased expression of MYC and E-cadherin by siRNA increased the invasiveness of MCF7 cells only when β3 integrin was expressed. The depletion of MYC and E-cadherin was assessed by western blot (**i**) and invasiveness was assessed by Boyden chamber assays (**j**; n=3). Results are expressed as mean± SD.