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News and Commentary

Bcl-2 together with PI3K p110α regulates cell morphology and cell migration

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The signaling network of phosphatidylinositol 3-kinase (PI3K) and Akt controls cell cycle, survival, metabolism and genomic instability. It is also involved in cell motility and cancer metastasis.[1] Genetic mutations of PI3K frequently happen in various cancers, induce PI3K dysfunction and result in increased cell migration and cancer metastasis.[2] Involvement of the PI3K-Akt pathway in the anti-apoptotic function of B-cell lymphoma 2 (Bcl-2) has been well defined. Previous work has also shown that activated PI3K results in phosphorylation of Akt, whereas the activated Akt in turn upregulates Bcl-2 by enhancing promoter activity of Bcl-2.[3] These findings suggest that PI3K is involved in the regulation of Bcl-2 expression. The mutation mediated dysfunction of PI3K may alter the regulation of Bcl-2. Our recent work, published in *Cell Death and Discovery*,[4] shows that Bcl-2 expression is downregulated at least threefold by the most frequent mutation H1047R in the p110α subunit of class IA PI3K. We show this by comparing endogenous levels of Bcl-2 in human colorectal cancer (CRC) HCT116 WT and MUT cells that were engineered from parental HCT116 cells to contain either the wild type (WT) or H1047R mutant (MUT)-p110α, respectively. This finding was further confirmed by the examination of PI3K p110α inhibition using PI3K inhibitor A66, which has greater specificity in inhibiting p110α as compared with other p110α inhibitors and thus maintains the function of other PI3Ks in growth factor signaling. Inhibition of H1047R-p110α results in an A66-dose-dependent increase in Bcl-2 expression. In contrast, inhibition of WT-p110α shows an A66-dose-dependent decrease in Bcl-2 expression.[4] These data suggest that cellular Bcl-2 levels are differentially regulated by the presence of either WT or MUT p110α.

In addition to its well-characterized role in the suppression of programmed cell death, Bcl-2 has been associated with cell proliferation, differentiation, mutagenesis, cytoskeletal reorganization, cell migration and cancer metastasis. Data examining the functional role of Bcl-2 in cell adhesion, migration and branching morphogenesis shows that lack of Bcl-2 in ureteric bud cells results in increased cell migration, increased cell invasion and decreased adhesion to vitronectin as compared with WT-ureteric bud cells, and suggests that Bcl-2 is required for the proper regulation of cell adhesive and migratory mechanisms, perhaps through modulation of the cellular microenvironment.[5] Another group examined the effects of Bcl-2 overexpression on cell morphology of undifferentiated PC12 cells and demonstrated that overexpression of Bcl-2 leads to disruption of the actin cytoskeleton and alteration of cell morphology.[6] Moreover, Ke *et al*. recently reported that overexpression of Bcl-2 inhibits cell adhesion, spreading and motility by enhancing actin polymerization.[7] They suggested that when overexpressed in both cancer and non-cancer cells, Bcl-2 can form a complex with actin and gelsolin that functions to decrease gelsolin-severing activity that leads to increased actin polymerization.

Actin polymerization can generate forces that underlie alterations in cellular morphology, protrusion, migration and chemotaxis that occur during morphogenesis.[8,9] Cancer cells control their migratory and invasive capability through morphogenic alteration. These processes involve a marked reorganization of the actin cytoskeleton and the concomitant formation of membrane protrusions required for cell motility in a complex three-dimensional environment, including lamellipodia, filopodia, podosomes and invadopodia.[10,11] Our data showed that the H1047R mutation in p110α of PI3K decreases actin polymerization, increases filopodia formation, and results in cell morphology changes in HCT116 cells (Figure 1a). Interestingly, H1047R mutation in p110α of PI3K downregulates Bcl-2, whereas the morphology of HCT116 MUT cells was altered when Bcl-2 was overexpressed (Figure 1b). Based on the aforementioned reports, the H1047R mutation mediated downregulation of Bcl-2 may provide an explanation for why the H1047R mutation in p110α can induce reorganization of actin cytoskeleton, and thus results in morphological changes and increased migratory capability in HCT116 MUT cells. The distinct effects of PI3K on regulation of Bcl-2 and actin cytoskeleton, however, resulted from either the presence of WT or MUT p110α. This suggests that WT and H1047R MUT p110α of PI3K may regulate actin cytoskeleton and cell migration by cooperation with Bcl-2 through distinct molecular mechanisms.

Overexpression of Bcl-2 occurs in many types of human cancers, and prevents cell death induced by nearly all anticancer drugs and radiation. The functional roles of Bcl-2 in tumor development and progression or metastasis, however, are quite unclear and often contradictory. Several

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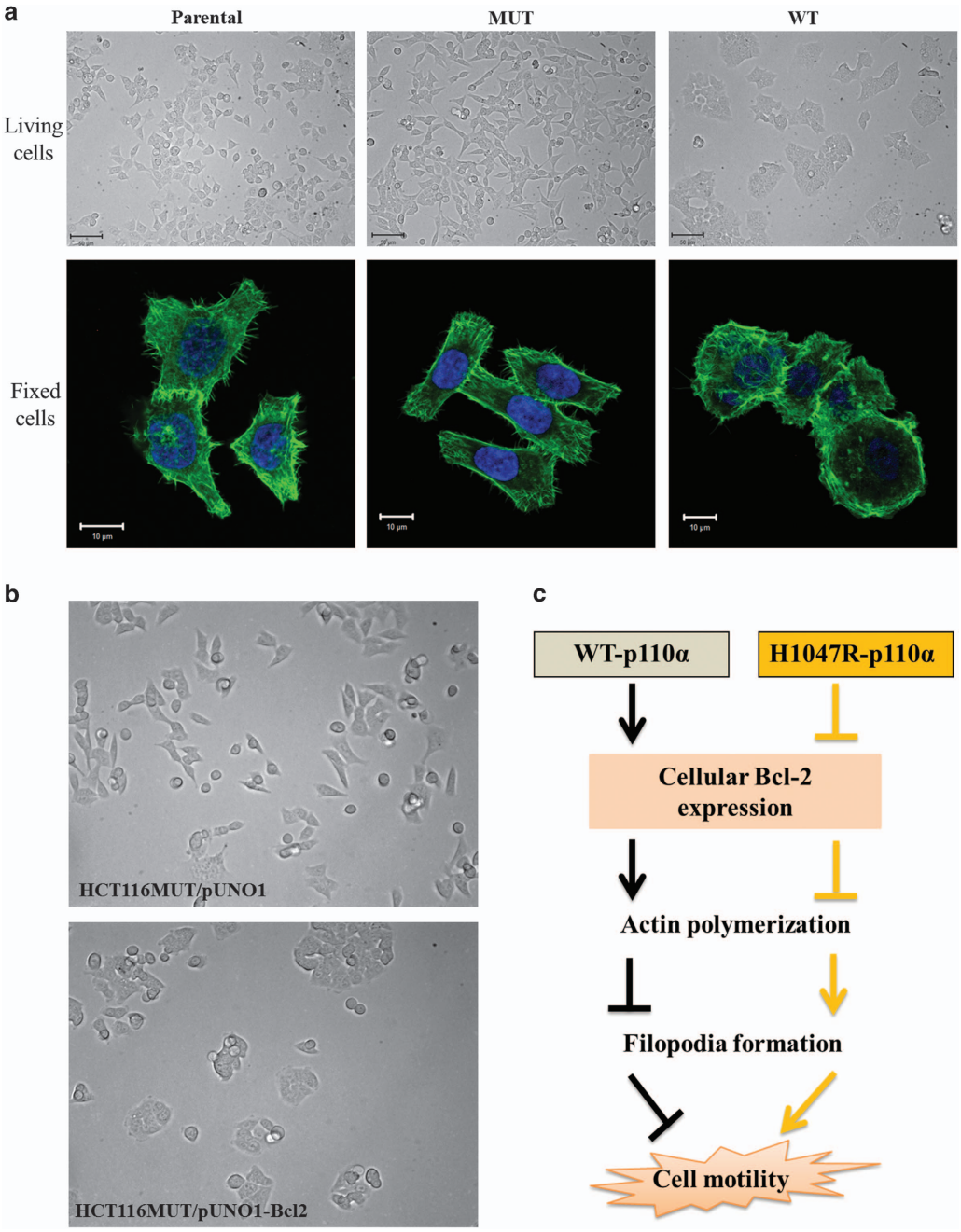

Figure 1 Cell morphology and cell migration of HCT116 cells are altered by the H1047R mutation in the p110α kinase domain of PI3K and Bcl-2. (**a**) Cell morphology of HCT116 cells. Top panel: cell morphologies of live parental, WT and MUT HCT116 cells captured at a 20 × magnification. Bottom panel: confocal images of parental, WT and MUT HCT116 cells captured at a 63 × magnification. Cells were fixed and stained for F-actin (green). Nuclei were stained with DAPI (blue). (**b**) Overexpression of Bcl-2 changed cell morphology of HCT116 MUT cells. HCT116 MUT cells were stably transfected with the pUNO1 or pUNO1-Bcl-2 plasmid and imaged at 20 × magnification, the morphology of cells changed as they became rounded and aggregated together when Bcl-2 was overexpressed. (**c**) Model for the cooperative role of Bcl-2 with WT or H1047R -p110α to control cell motility in HCT116 cells. The symbol ⊥ means decrease and ↓ means increase. The H1047R mutation in p110α causes the downregulation of Bcl-2, which decreases actin polymerization, induces reorganization of actin cytoskeleton

reports have indicated that Bcl-2 increases tumor progression in some types of cancer. On the other hand, data from previous *in vivo* studies have shown that loss of Bcl-2 expression correlates with tumor recurrence in CRC[12] and high levels of Bcl-2 are predictive of relapse-free survival in stage II CRC.[13] Clinical observations reporting that Bcl-2 expression in breast cancer can be associated with a favorable prognosis suggests a possible beneficial role for Bcl-2 in suppressing tumor progression and metastasis.[14] Using an *in vitro* wound healing assay, we showed that H1047R-p110α increases migratory capacity of HCT116 cells. The cell migration, however, was slowed down in HCT116 MUT cells when Bcl-2 was stably overexpressed.[4] To note, a recent study shows that knock-down of Bcl-2 proteins directly inhibits the migration and

invasion of the CRC cells HT29 and SW480, independent of their cell death induction or effects on proliferation.[15] These contradictory effects of Bcl-2 overexpression on cell migratory capability seen in different CRC cell lines may indicate the importance of cellular environment, for example the presence of different types of PI3K p110*α*. It is known that SW480 cell line expresses WT PI3K and that HT29 cells bear the $P449T^{b}$ mutation in p110*α*. All of these observations further suggest the multiple and complex functions of Bcl-2 and PI3K.

In conclusion, although Bcl-2 functions as an oncogene to prevent programmed cell death and promotes tumorigenesis, our study has shown that high levels of Bcl-2 may also prevent tumor metastasis. This function is probably due to the ability of Bcl-2 to regulate actin polymerization in a way that inhibits cell migration. Moreover, Bcl-2 may be differentially regulated by PI3K depending on the presence of WT or MUT p110*α* that may activate distinct signaling pathways and differentially control cell migratory capability and cancer metastasis (Figure 1c). Our work links the MUT and WT types of PI3K p110*α* and Bcl-2 in controlling cytoskeleton rearrangement, migratory capability of CRC cells and CRC metastasis. This may provide a novel concept for performing studies on molecular mechanisms involved in cancer metastasis and a possible biomarker development for predicting cancer metastasis.

Conflict of Interest

The authors declare no conflict of interest.

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