Distinct roles of class I PI3K isoforms in multiple myeloma cell survival and dissemination

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Distinct roles of class I PI3K isoforms in multiple myeloma cell survival and dissemination

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The phosphoinositide 3-kinase (PI3K) pathway has a crucial role in tumor progression and drug resistance, including both conventional chemotherapeutics as well as novel agents.1 Although no mutations have been described in the PI3K/Akt genes in multiple myeloma (MM), it was shown that this pathway is constitutively activated in MM cells and has pleiotropic effects influencing proliferation, drug resistance, angiogenesis and cell adhesion.2

PI3Ks are divided into three subclasses, and of these, class I PI3Ks—p110α (also known as PIK3CA), p110β (also known as PIK3CB), p110γ (also known as PIK3CG) and p110δ (also known as PIK3CD)—are well described in terms of their role in cancer development and progression.3,5 PIK3CA is frequently mutated in solid tumors including carcinoma of the prostate, breast colon and endometrium.4,5 However, there have been no reports of cancer-specific mutations in MM.6

Recently, a number of potential therapeutics targeting specific PI3K groups or isoforms were developed.4,6 Previous studies have indicated that p110α, p110β and p110δ might be potential targets for MM.7–9 Although the basic framework of PI3K signaling has been uncovered, the contribution of the different PI3K isoforms is not well understood.8 In the current study, we investigated the functional role of class I PI3K isoforms in modulating MM cell trafficking in vivo and in vitro.

To examine activation of the PI3K/Akt pathway in MM, we first performed gene set enrichment analysis10 on the gene-expression data set (Shaughnessy et al. ref. GSE24080) of patients in different International Staging System stages of MM compared with normal development and progression.1,3 PIK3CA is frequently mutated in MM cells to primary MM-derived BM-MSCs; and found that by inhibiting p110α, p110β and p110δ, cell cycle progression was significantly lower in p110α- and p110δ-knockdown cell-injected mice compared with scramble cell-injected mice (P<0.05); whereas tumor growth observed in p110α- and p110γ-knockdown cell-injected mice was similar to control mice (Figures 2a and b). We speculate that this might be due to markedly decreased tumor cell growth triggered by MM cell adhesion to BM-MSCs, as the adhesion of MM cells to BM-MSCs activates many pathways and has a vital role in MM pathogenesis and disease progression.12 We further confirmed that tumor cells showed knockdown for each p110 isoform, as demonstrated ex vivo on tumor cells harvested from each cohort of mice (Figure 2c). Mice were followed until the development of hind limb paralysis or death, and Kaplan–Meier analysis was performed showing prolonged survival in all groups except p110α mice (p110β and p110γ, P<0.05; p110α, P>0.001; Figure 2d). Despite similar tumor burden observed between p110γ mice and scramble control-injected mice, mice injected with p110γ knockdown cells had improved survival compared with control mice. This might be due to the different extent of tumor involvement of various organs13 between the two groups, thus explaining the differences in survival.

Interestingly, our data indicate that p110α is not critical for the survival of MM cells in vivo. Unlike most solid tumor malignancies, where PI3KCA (p110α) mutation is the leading cause of activation of this pathway and is the target of many therapeutic agents in development,3 there have been no reports of this specific mutations in MM.6 Moreover, it was shown that unlike wild-type p110α, overexpression of the wild-type p110β, p110γ and p110δ is sufficient to induce an oncogenic transformation of fibroblasts in cell culture.14

In this study, p110β was highly expressed in all MM cell lines, whereas only a minor subset expressed p110δ at the protein level (Figure 1b), which is consistent with a recent report9 showing expression of p110β in 38 MM cell lines in comparison to the detectable expression of p110δ in only 4 cell lines. In addition, another study9 reported similar findings in cell lines showing lack of p110δ expression in most MM cell lines. Of note, we found discrepancies in p110δ expression in cell lines between our study and prior published studies but our data was confirmed in the Cancer Cell Line Encyclopedia data at the mRNA level (data not shown).15 Importantly, Ikeda et al.15 evaluated p110δ levels in patient samples and detected its expression in all 24 MM patients. This may provide a clinical rationale for targeting p110δ despite the lack of expression of p110δ in MM cell lines.

Overall, our data suggest that, in contrast with solid tumors, MM may be more dependent on PI3K p110β and p110δ and less dependent on PI3Kα, and these may be the focus of drug development in this hematological malignancy.

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Figure 1. The role of class I PI3K-mediated Akt signaling in MM. (a) Gene set enrichment analysis software analyzed functionally related genes in class I-mediated Akt activation with statistically significant enrichment (false-discovery rate q-values < 0.25; < 0.25 is considered significant), using gene-expression data set (GSE24080). Plots show enrichment results for the gene set (left, stage I MM vs normal subjects; middle, stage II MM vs normal subject; right, stage III MM vs normal subjects). (b) Baseline expression of the different PI3K isoforms (p110α, β, γ and δ) in MM cell lines was detected by immunoblotting using isoform-specific antibodies. MM tumor cells (MM.1S-GFP+/Luc+) were infected with lentivirus-mediated small hairpin (sh)RNA. Reverse transcription quantitative PCR (c) and immunoblotting (d) were performed to show infection efficiency and isoform specificity, respectively. Scramble and knockdown tumor cells (p110α, β, γ and δ) were cocultured with BMSCs overnight, and MM cells were then separated from the BMSCs, lysed and whole-cell lysates were subjected to immunoblotting with Akt and P-Akt (Thr308 and Ser473), which shows decreased phosphorylation of Akt in knockdown cells. The effects of inhibition of PI3K isoforms by shRNAs on cell survival were assessed by 3-(4,5-dimethylthiazol-2-yl)/2,5-diphenyltetrazolium bromide (MTT) assay (f). Adhesion assay (g) was performed to show the ability of knockdown cells to adhere to BMSCs after 2 h of incubation.
CONFLICT OF INTEREST

IMG is on the advisory board for Onyx, BMS and Celgene, and receives research lab support from Genzyme and BMS. The remaining authors declare no conflict of interest.

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AUTHOR CONTRIBUTIONS

IS: designed and performed the research, analyzed the data and wrote the manuscript; MM, YM, SVG, BT, FA, YZ, PM, AS, AKA and AMR: performed the research and analyzed the data; IMG: supervised the study and wrote the manuscript.

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Figure 2. Knockdown of PI3K isoforms regulates tumor progression and survival in vivo. MM.1S-GFP+/LacZ tumor cell lines (Scr, p110 alpha, beta, gamma and delta) were injected intravenously into SCID-Bg mice and tumor growth was assessed by in vivo bioluminescence imaging (BLI). (a) Representative BLI of each group in different time points is shown. (b) Quantification of BLI signals demonstrated that p110 beta and delta mice showed significant reduction in tumor growth (P<0.05) compared with scramble mice. (c) Reverse transcription quantitative PCR was performed on tumor cells that were harvested from hind leg bones of animals by bone marrow flushing. (d) Survival of mice was evaluated until complete hind limb paralysis or death using Kaplan-Meier curves. Compared with scramble mice, all groups except p110 alpha showed prolonged survival (p110 beta and gamma, P<0.05; p110 delta, P<0.001).


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