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Letter to the Editor

Cancer Research

Bcl-2 Protein Targeting by the p53/p21 Complex—Letter

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Kim and colleagues reported a p53/p21 complex regulates cancer cell invasion and apoptosis by targeting the Bcl-2 family (1). Interaction of overexpressed p53 and p21 proteins in p53-null H1299 cells correlated with decreased cell invasion. No coimmunoprecipitation experiments documenting endogenous p53:p21 protein interaction were performed. There was no consideration of how the p53/p21/Bcl-w complex might relate to p53/MDM2 or p21/cyclin/CDK/PCNA complexes. The authors showed similar findings in IMR-32 cells that carry cytoplasmic wild-type (wt-p53). Although they demonstrated si-p53 or si-p21 affect Bcl-w:Bax interaction, they did not prove endogenous p53 interacts with p21.

There was a modest effect of p53 on suppression of invasion (Fig. 1; ref. 1), and this phenotype was suppressed by si-p21. However, there was no effort to determine whether effects on invasion could be explained by altered cell-cycle phase or proliferation state. This is important as tumor cell growth and proliferation is required for invasiveness (2). On the other hand, some evidence suggests growth arrest may be required for invasion (3). Kim and colleagues state that cell invasion is regulated by p53/p21, but little insight is provided beyond cell-cycle and growth regulation. The authors suggest suppression of invasion is mediated by Bax. However, Bax is a direct target of p53, and its effects on apoptosis are well established (dead cells do not invade). The authors do not consider direct regulation of p53 toward Bax and whether this could explain their findings. Endogenous p53 decreases invasion by promoting MDM2:Slug interaction, resulting in Slug ubiquitin–mediated degradation (4). The authors did not experimentally consider these mechanisms.

Kim and colleagues show results inconsistent with the paradigm in the p53 field. First, WT-p53 is stabilized following exposure of cells to DNA damage such as ionizing radiation (5). In Fig. 5B of ref. 1, lane 6 shows no increase in nuclear p53 expression following 20 Gy of radiation; rather, p53 remains cytoplasmic. Second, it is well known that overexpression of p53 suppresses cancer cell growth. Figure 6 of ref. 1 shows similar tumor growth of exogenously transfected wt-p53 versus vector control and greater than p53ΔC37 at 0 Gy. These unexpected results should be better documented, including WT-p53 localization by immunofluorescence and WT-p53 functionality *in vivo*, to validate whether these are cell type–specific phenotypes. Overexpression of p53ΔC37 was not characterized, and the mutant lacks one of two nuclear localization signal. Whether effects are due to failure of p21 interaction or inability of p53 nuclear import to activate transcription of p53 targets was not addressed.

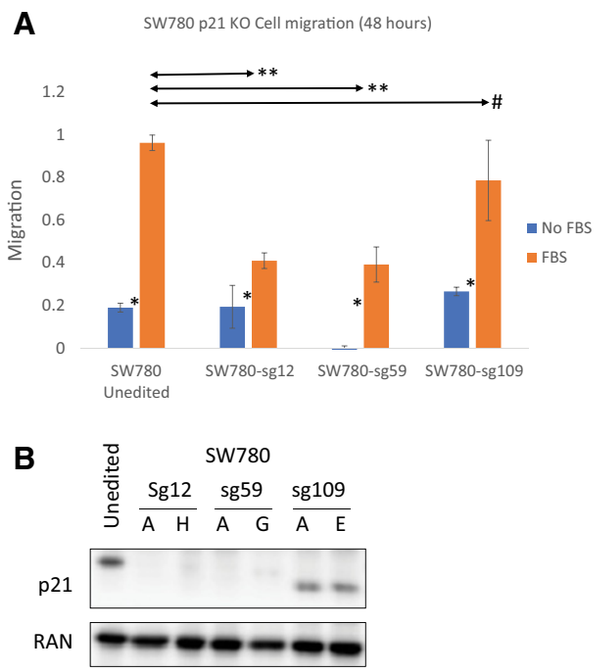

Figure 1.
p21 ablation or mutation does not increase cell migration that is needed for invasion. **A,** p21 knockout cell lines were generated using pLentiCRISPR-E (Addgene #78852). The xCelligence system was used to quantitate mobility of unedited SW780 cells and single-cell clones (clone A from each guide) with indels in CDKN1A at amino acids 12, 59, or 109 in the presence or absence of FBS. Plotted is the absolute migration ± one SD. Migration between unstimulated and FBS-stimulated cells served as a positive control for each cell line. *, $P < 0.002$ for all cell lines, Student *t* test. FBS-stimulated migration was compared between unedited cells and each p21 knockout cell line. **, $P < 0.0001$; #, $P = 0.18$, Student *t* test. **B,** Western blot analysis showing that p21 protein is abolished in the sg12 and sg59 clones, but that a truncated peptide is retained in the sg109 clones. SW780 cells were obtained from ATCC, authenticated by STR sequencing analysis, and PCR tested as *Mycoplasma* free.

p21/CDKN1A is not infrequently mutated in bladder cancer (The Cancer Genome Atlas; ref. 6). We used p21-CRISPR in bladder cancer cells to directly test the hypothesis that p21 mutation might contribute to greater migration or invasiveness of tumor cells. CRISPR knockout of CDKN1A resulted in decreased cellular motility (Fig. 1A). This effect was muted when indels were targeted to the C-terminus of the protein, when a truncated peptide was still detectable and retaining the CDK-interacting domain (Fig. 1B). Importantly, SW780 cells carry WT-p53, and 50% of bladder cancers retain two intact copies of TP53. Thus, p21 ablation or mutation does not increase cell migration that is needed for invasion.

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p21 appears to protect from cell death through various mechanisms (6), including growth arrest and cytoplasmic effects of p21. The role of p21 in apoptosis following p53 overexpression in H1299 cells in Kim and colleagues should have been better substantiated as a cell type–specific phenotype.

For multiple reasons, the evidence supporting the model put forth by Kim and colleagues is lacking in rigor and is not convincing due to alternative explanations. Kim and colleagues reported similar findings that have similar limitations (7, 8). It is important to document endogenous protein:protein interactions and to include critical controls to support novel paradigm-shifting results. It remains unclear whether a physiologic complex of p53/p21/Bcl-w regulates invasion or apoptosis.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

1. Kim EM, Jung CH, Kim J, Hwang SG, Park JK, Um HD. The p53/p21 complex regulates cancer cell invasion and apoptosis by targeting Bcl-2 family proteins. Cancer Res 2017;77:3092–100.
2. Iwasaki T, Shinkai K, Mukai M, Yoshioka K, Fujii Y, Nakahara K, et al. Cell-cycle- dependent invasion in vitro by rat ascites hepatoma cells. Int J Cancer 1995;63:282–7.
3. Kohrman AQ, Matus DQ. Divide or Conquer: cell cycle regulation of invasive behavior. Trends Cell Biol 2017;27:12–25.
4. Wang SP, Wang WL, Chang YL, Wu CT, Chao YC, Kao SH, et al. p53 controls cancer cell invasion by inducing the MDM2-mediated degradation of Slug. Nat Cell Biol 2009;11:694–704.
5. Kastan MB, Onyekwere O, Sidransky D, Vogelstein B, Craig RW. Participation of p53 protein in the cellular response to DNA damage. Cancer Res 1991;51:6304–11.
6. El-Deiry WS. p21(WAF1) mediates cell-cycle inhibition, relevant to cancer suppression and therapy. Cancer Res 2016;76:5189–91.
7. Kim EM, Park JK, Hwang SG, Kim WJ, Liu ZG, Kang SW, et al. Nuclear and cytoplasmic p53 suppress cell invasion by inhibiting respiratory complex-I activity via Bcl-2 family proteins. Oncotarget 2014;5:8452–65.
8. Kim J, Bae S, An S, Park JK, Kim EM, Hwang SG, et al. Cooperative actions of p21WAF1 and p53 induce Slug protein degradation and suppress cell invasion. EMBO Rep 2014;15:1062–8.

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