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651.MULTIPLE MYELOMA AND PLASMA CELL DYSCRASIAS: BASIC AND TRANSLATIONAL

Synergistic Anti-Tumor Effects in RAS ^{Mut} Multiple Myeloma By Targeting MAP4K2 and RAS Pathways

Jing Fu, PhD[1], *Shirong Li, PhD*[2], *Guifen Liu*[3], *Huihui MA*[4], *Christophe Marcireau*[5], *Markus Mapara, MD*[6], *Suzanne Lentzsch, MD PhD*[7]

[1] Columbia University Medical Center, New York, NY
[2] Columbia University Irving Medical Center, New York, NY
[3] Columbia University Irving Medical Center, NEW YORK, NY
[4] Columbia Center for Translational Immunology, New York, NY
[5] Sanofi, Paris, France
[6] Blood and Marrow Stem Cell Transplantation Program, Division of Hematology & Oncology, Columbia University Irving Medical Center, New York, NY
[7] Division of Hematology & Oncology, Columbia University Medical Center, New York, NY

Mitogen-activated protein kinase kinase kinase kinase 2 (MAP4K2) is an important key regulator of the stress-activated MAPK core signaling pathways. Our previous work demonstrated that MAP4K2 is highly expressed in multiple myeloma (MM) cells, and MM cells carrying a RAS mutation are more vulnerable to MAP4K2 inhibition, associated with the downregulation of critical transcriptional factors including IKZF1/3, BCL-6, and c-MYC proteins (*Li et al. Blood 2021*). Targeting the aberrant RAS/MAPK signaling, MEK inhibitors have been shown to induce cytotoxicity in MM (De la Puente, P. *et al. Blood Cancer J. 2016*). Thus, we used a recently developed MEK inhibitor (MEKi) Trametinib to investigate the therapeutic potential of combined MEK and MAP4K2 signaling inhibition in RAS-driven MM.

Tet-on sh-MAP4K2 lentivirus was introduced into RAS ^{Mut} MM cells to establish the inducible MAP4K2 knockdown cells upon doxycycline treatment. To examine the combined effects, Tet-on sh-MAP4K2 RAS ^{Mut} MM cells were treated with doxycycline to silence MAP4K2 expression, followed by treatment of MEKi Trametinib at different dosages. Cell proliferation, apoptosis, and cell cycle were analyzed five days later. Data showed that MAP4K2 silencing combined with MEK inhibition significantly decreased cell proliferation to 10% compared with MEKi alone 32% ($p<0.01$) by MTS assays, and enhanced cell apoptosis (MEKi alone vs. with MAP4K2 silencing: 26% vs 91%, $p<0.01$). Cell cycle assay demonstrated that MEKi induced G1 arrest in RAS ^{Mut} MM cells (MEKi alone:76% vs vehicle control: 54%, $p<0.01$), which was also significantly higher in combination with MAP4K2 silencing (92%). Western blot assay confirmed that MAP4K2 silencing combined with MEKi significantly enhanced the downregulation of IKZF1 and c-MYC compared to the MEKi treatment alone. These data suggest that the MEKi and MAP4K2 inhibition combination has synergetic anti-MM effects.

To further confirm the synergetic effects, we tested the combined effects of MEKi with the MAP4K2 kinase inhibitor TL4-12. H929 (N-Ras ^{Mut}) cells were treated by vehicle control, TL4-12 (2 uM) alone, MEKi (200 nM) alone, or TL4-12 + MEKi for 4 days, followed by an apoptosis assay. The combination of TL4-12 and MEKi strongly enhanced the cytotoxicity effects compared to the single-agent treatments. Consistently, the carboxyfluorescein diacetate succinimidyl ester (CFSE) cell proliferation assay confirmed that dual treatment significantly delayed H929 cell proliferation compared to the single-agent treatments (percentage of proliferating cells: TL4-12 alone: 86.2%; MEKi alone: 89.03%; dual treatment: 18.9%. $p<0.01$). Western blotting assay further confirmed that dual treatment of MEKi and MAP4K2i enhanced the decrease in MEK1/2 and ERK1/2 phosphorylation and downregulation of transcriptional factors IKZF1 and c-MYC.

Taken together, our findings demonstrate that the combination of MAP4K2 inhibition with MEKi results in synergetic anti-MM effects in RAS mutation myeloma, therefore could be a potent novel therapeutic regimen for patients with relapsed/refractory multiple myeloma.

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