Introduction

Melanoma remains the most lethal form of skin cancer, exhibiting high morbidity rates due to a high likelihood of developing metastases and acquiring drug resistance. Approximately 40-50% of melanomas contain oncogenic BRAF mutations, of which the most common is the BRAF V600E mutation. This mutation constitutively activates the mitogen-activated protein kinase (MAPK) signaling pathway, leading to uncontrolled cell growth and oncogenesis. Recent combination therapies of BRAF- and MEK-specific inhibitors have shown improved progression-free patient response in phase II clinical trials. However, in a majority of patients, acquired resistance to MAPK pathway inhibiting therapies develops after approximately 12 months of treatment. Preclinical studies have suggested that MEK1 mutation confers resistance to BRAF and MEK inhibitors. In this study, we used the CRISPR/Cas9 genome-editing technology to generate a drug resistant MEK1Q56P knock-in mutation within the A375 melanoma cell line which naturally harbors the BRAFV600E mutation. We validated this isogenic cell model using both molecular and biofunctional approaches.

Background

Role of mutant BRAF in preventing apoptosis and driving melanoma cell survival

Results

Design and construct CRISPR/Cas9 regents to generate the MEK1Q56P mutation in the human melanoma A375 cell line

Figure 1. Retrograde BAX drive the phosphorylation and subsequent proteasomal degradation of BAK and Bax via the MEEK pathway. In the absence of BRAF, the anti-apoptotic proteins Bcl-2 and Bcl-xl are not inhibited, leading to increased cell survival. When the BRAF pathway is blocked through the use of BRAF inhibitors, the pro-apoptotic occurs.

Confirmation of MEK1Q56P mRNA expression in single cell clones

Figure 2. BRAFV600E melanoma cells treated with BRAF inhibitors acquire drug resistance by switching between ARAF, BRAF, and CRAF (isoforms of BRAF), leading to the activation of the MAPK pathway. Drug resistance can also occur through the MEK1Q56P mutation. This mutation is observed in NIH60 (GEM) and A375 (GEM). Furthermore, activation of the MEK1Q56P pathway could give the additional resistance from increased GR1 and PDGFR signaling.

MEK1Q56P mRNA in cell clones carrying the GSPS mutation. (A) Screening for the MEK1Q56P mutation in recombinant clones was done using DNA gel from cells and performing PCR (red arrow). (B) Gel image of the PCR products. (C) The reinsertion of the MEK1Q56P point mutation into the cell line was confirmed by Sanger sequencing on the right. The expected point mutation is boxed in red.

Figure 3. Schematic of the target region for integration of the CRISPR/Cas9 sequence. The Cas9 transcriptional activation construct was delivered to melanoma cell line. The inserted Cas9 target site was subsequently validated by insertion and integration of the MEK1 Q56P sequence by either homology-directed repair (HDR) or non-homologous end joining (NHEJ) at the two sgRNA-Cas9 cut sites.

PCR and sequencing to confirm the MEK1Q56P knock-in mutation

Figure 4. Confirmation that Cas9-mediated genome editing and mutation integration in recovered clones was carried out using PCR primers as shown left, with the gel image of PCR products on the right. (A) Introduction of the MEK1Q56P point mutation into the cell line was confirmed by Sanger sequencing primers shown in (B). Amplified bands expected and indicated in red. (C) Confirmation of the MEK1Q56P knock-in mutation in recovered clones.

Conclusion

The ATCC NCI60 assay A375 cell line (ATCC® CRL-1619™) derived from the A375 melanoma cell line (ATCC® CRL-1619™) contains the MEK1Q56P mutation. This have been validated at the genomic and transcript levels. This cell line allows for the testing of MEK1Q56P isogenic line's resistance to BRAF inhibitors. Parental A375 cells and MEK1Q56P isogenic clone were treated with dabrafenib, trametinib, MEK1 inhibitor, MEK1/2 inhibitor and non-specific chemotherapy drugs were similar. The expression of MEK1Q56P in recovered clones was confirmed via Sanger sequencing. This observation was further validated in (B) when specific concentration of each drug and combination of BRAF and MEK1 inhibitors demonstrated the synergistic effect of BRAF and MEK inhibitors, which was indicated by the survival rate of the parental A375 cell line (B, C and D, E,F respectively). The responses of the MEK1Q56P isogenic line is resistant to BRAF & MEK1 inhibitors demonstrated the synergistic effect of treatment. This is likely due to the MEK1Q56P isogenic line's resistance to BRAF inhibitors. Parental A375 melanoma cell line and the MEK1Q6P isogenic line are similar. This have been validated at the genomic and transcript levels.

References