Specific BaF3 tumor platform for c-KIT and KRAS mutations

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Abstract Number:6960

Abstract

The c-KIT oncogene is located on chromosome band 4q12 and encodes a type III RTK family transmembrane glycoprotein, whose ligand is stem cell factor (SCF). The binding of SCF promotes c-KIT dimerization and transphosphorylation, activating downstream signaling pathways involved in proliferation, differentiation, migration, and survival. In human cancer, KRAS gene mutation occurs in nearly 90% of pancreatic cancer, 30-40% of colon cancer, 17% of endometrial cancer and 15-20% of lung cancer (mostly NSCLC). Research has found that KRAS mutations are mainly located at four hotspot codons (12, 13, 61, and 146). Codon 12 has the highest mutation frequency among all four hotspot codons, with G12D mutation being the most common, followed by G12V, G12C, and others. Ba/F3 is a mouse pro-B cell line that relies on interleukin-3 for survival and can also be introduced with exogenous activated kinases for growth. Ba/F3 cells are widely used as models for evaluating the efficacy of kinase oncogenes, downstream signal transduction, and the blocking ability of small molecule kinase inhibitors. We have constructed over 300 stable Ba/F3 point mutant strains and conducted in vivo xenograft experiments on some of them, obtaining good tumorigenic and pharmacological data. In this study, we constructed CDX models using BaF3 c-KIT mutant cell lines and BaF3 KRAS-G12C mutant cell lines. The results showed that the BaF3 mutation tumor models are suitable for the drug discovery of specific point mutation anti-cancer drugs.

Methodology

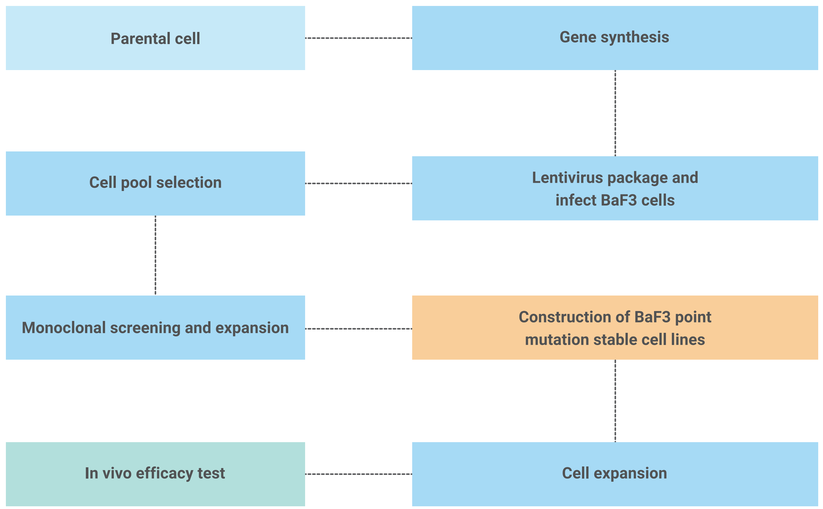


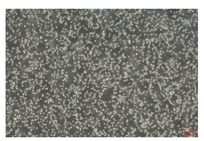
Figure 1: Schematic diagram of establishment of BaF3 point mutation stable cell lines

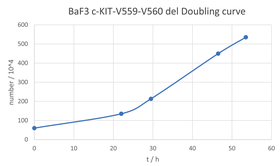
Results

1.BaF3 c-KIT mutation tumor models

Morphology and DT of BaF3 c-KIT-V559-V560 del, and BaF3 c-KIT-W557-K558-T670I del

BaF3 c-KIT-V559-V560 del





BaF3 c-KIT-W557-K558 del-T670I

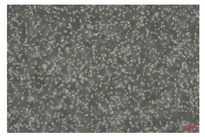
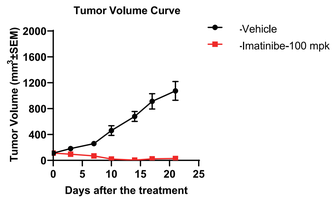
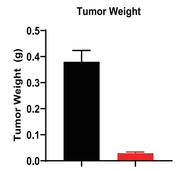


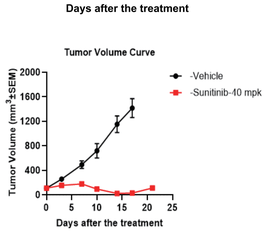
Figure 2: Optical microscope image and doubling curves of BaF3 c-KIT-V559-V560 del and BaF3 c-KIT-W557-K558 del-T670I.

The doubling times of BaF3 c-KIT-V559-V560 del and BaF3 c-KIT-W557-K558 del-T670I are 16.95 and 16.76 hours, respectively, which is close to the parental cell doubling time of 20 hours.

In vivo efficacy evaluation of BaF3 c-KIT mutation tumor models







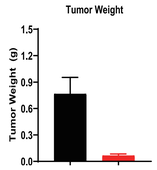
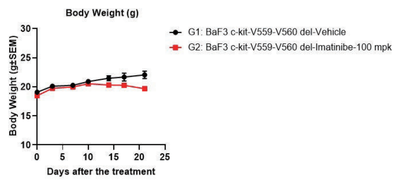


Figure 3: BaF3 c-KIT-V559-V560 del and BaF3 c-KIT-W557-K558 del-T670I tumor xenograft growth curves and comparison of BaF3 c-KIT-V559-V560 del and BaF3 c-KIT-W557-K558 del-T670I tumor weight after drug administration respectively.

For the in vivo pharmacological experiment of BaF3 c-KIT-V559-V560 del cell, the average tumor volume of the solvent control group mice was 911.87 ± 119.10 mm3, and the average tumor volume of the positive drug Imatinibe-100 mpk mice was 24.48 ± 4.15 mm3 (TGI was 111.10%, P value<0.0001). There were significant differences between the positive drug Imatinibe-100 mpk group and the solvent control group. For the in vivo pharmacological experiment of BaF3 c-KIT-W557-K558 del-T670I cell, the average tumor volume of the solvent control group mice was 1414.24 ± 153.85 mm3, and the average tumor volume of the positive drug Sunitinib-40 mpk mice was 28.92 ± 2.75 mm3 (TGI was 106.13%, P value was 0.0054). There were significant differences between the positive drug Sunitinib-40 mpk group and the solvent control group.

Changes in body weight after drug administration testing



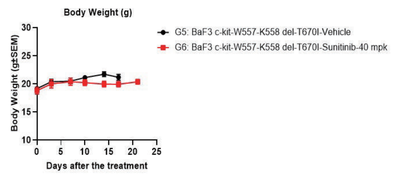


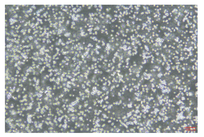
Figure 4: Body weight change curves of BaF3 c-KIT-V559-V560 del and BaF3 c-KIT-W557-K558 del-T670I tumor models.

The mice are in good condition, and there is no statistical difference in weight changes between groups.

2.BaF3 KRAS-G12C mutation tumor models

Morphology and DT of BaF3 KRAS-G12C

BaF3 KRAS-G12C



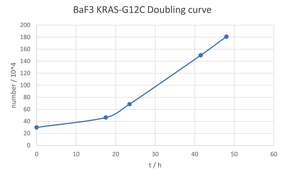
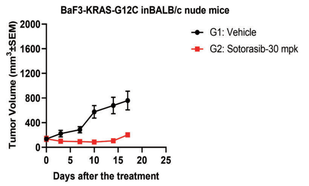


Figure 5: Optical microscope image and doubling curves of BaF3 KRAS-G12C.

The doubling time of BaF3 KRAS-G12C is 18.51 hours, which is close to the parental cell doubling time of 20 hours.



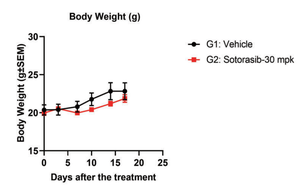


Figure 6: Tumor xenograft growth curve and body weight change curve of BaF3 KRAS-G12C.

For the in vivo pharmacological experiment of BaF3 KRAS-G12C cell, the average tumor volume of the solvent control group mice was 759.27 ± 151.35 mm3, and the average tumor volume of the positive drug Sotorasib-30 mpk mice was 202.92 ± 24.96 mm3 (TGI was 89.37%, P value was 0.0222). There were significant differences between the positive drug Sotorasib-30 mpk group and the solvent control group.

Discussion

BaF3 c-KIT-V559-V560 del, BaF3 c-KIT-W557-K558 del-T670I and BaF3 KRAS-G12C exhibited good tumorigenic effects: fast tumorigenesis rate and uniformity of tumorigenesis. The tumor in the positive drug group showed a downward trend, and the tumor inhibition effect was good. The tumor weight analysis results showed that the positive compound exhibited significant tumor inhibitory effects on both BaF3 c-kit-V559-V560 del and BaF3 c-kit-W557-K558 del-T670I. In addition, the mice are in good condition, and there is no statistical difference in weight changes between groups. In conclusion, the BaF3 mutation tumor models we constructed can provide accurate preclinical pharmacological research services for relevant targets, and validate anti-tumor activity through positive drug group testing, providing stable and reliable model data.

Summary

In the BaF3 tumor transplantation mouse model carrying c-KIT/KRAS-G12C targets, we set up a positive drug group (Imatinib, Sunitinib or Sotorasib), and calculated TGI (%), T/C (%), and RTV based on changes in tumor volume, mouse body weight, and tumor weight. The results showed that Imatinib, Sunitinib or Sotorasib exhibited certain anti-tumor activity. In summary, the BaF3 point mutation stable cell lines have the ability to test kinase mutations, analyze candidate drug and compound libraries, and predict clinical drug resistance.

References

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