Subcutaneous and orthotopic patient-derived human hepatocellular carcinoma xenograft models for pharmacodynamic efficacy assessment of sorafenib

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Abstract

The development of oncology drug needs a lot of money and manpower from preclinical study to approach. To resolve this "Valley of Death", a reliable and predictable animal model to evaluate the pharmacodynamic effects of new candidate is needed. There has been a recent increase in the use of patient-derived tumor tissue (PDTT) xenografts (PDX) engrafted into immune-compromised mice for preclinical modeling. The PDTT xenograft tumor models retained histology, genome and biology similarities compared with their donors, and could be used as a more accurate screening tool and evaluate key markers of responses to novel drugs. We minced primary human hepatocellular carcinoma (HCC) tissues, mixed them with matrigel, and injected subcutaneously and orthotopically into immunodeficient mice. One primary tissue from a 59-years-old male patient grown and allowed a subcutaneous passage in the second mouse after serial transplantation and also showed similar histologic presentation to original donor tumor. Comparative analysis by gene chips also showed genomic similarity in different passaged tumors. The primary HCC xenograft of subcutaneous and orthotopic models were used to evaluate the pharmacodynamic effects of sorafenib. Our results demonstrated that sorafenib inhibited 59.4% tumor growth and reduced 52.6% secretion of human alpha-fetoprotein (h-AFP) in subcutaneous model. In addition, sorafenib also reduced 83.3% secretion of h-AFP and prolonged the survival of orthotopic HCC mice. We successfully established a new HCC xenograft tissue line from PDTT and can be used to evaluate the new anti-HCC candidates and applied to achieve individualized chemo-drugs by preclinically assessing.

Materials and Methods

Animals

C.B-17 SCID mice, female, 6-8 weeks. Weighted about 18-20g.

Tumor source

The HCC tumor was derived from 59-years-old male(Pt16, HCV+), and then implanted subcutaneously in SCID mice (P0).

Subcutaneous and orthotopic implantation

Mice are anesthetized with Zoletil/Xylazine solution [40mg/kg Z+10mg/kg X, I.M.] and the minced grown tumor (P0) was mixed with matrigel and implanted subcutaneously by trocar. Left lateral lobe liver of the anesthetized mice was exposed following an upper middle incision and tumor fragments were mixed with matrigel and injected into liver directly.

h-AFP determination

Serum was collected by submandibular bleeding or cardiac puncture, and serum h-AFP level were determined by ELISA kit (DAFP00, R&D).

Therapeutic experiments

Subcutaneous and orthotopic model mice were treated with sorafenib (30 mg/kg B.W./day, P.O.).

Statistical analysis

Student t-test was applied for tumor growth and Log-Rank for survival in this study. The $p < 0.05$ was considered to be statistically significant.

Results

Table 1. Clinical information of Pt16 HCC tumor.

Number	Age	Gender	Virus	α-FP	Stage	P1 v.s. P4 (HT12 gene chip)
16	59	male	HCV	2434.3	pT2	0.9885

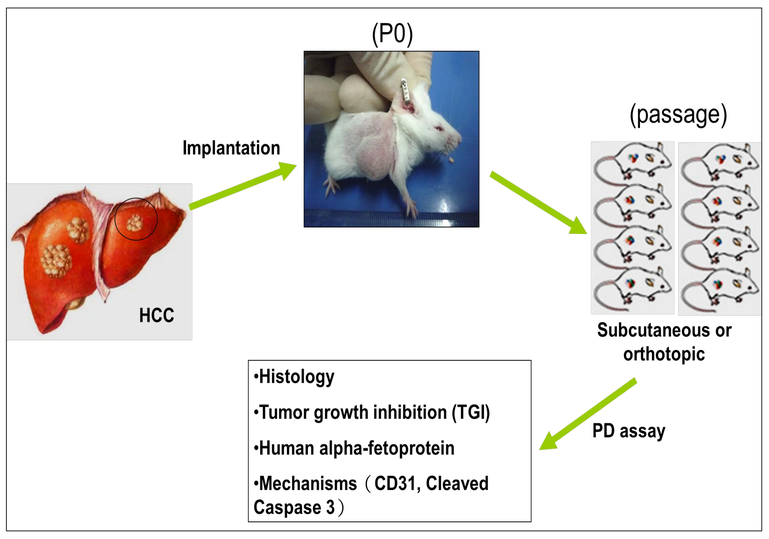

Fig 1. Flowchart of this study.

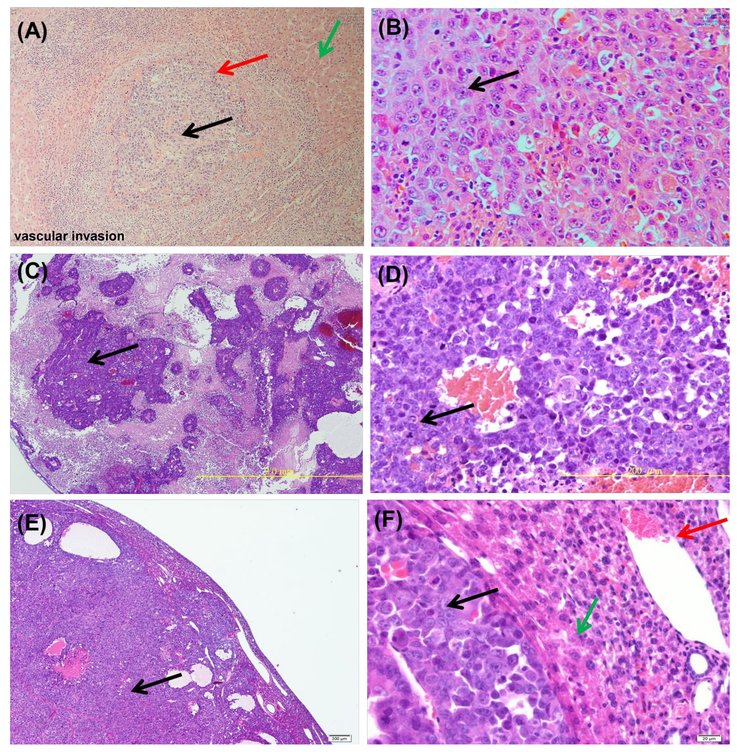

Fig 2. Histology of passage tumors and donor tissue. H&E staining. (A, 100X) donor tumor, vascular permeation. (B, 400X) donor tumor and moderately differentiated. (C, 40X) Subcutaneous tumor. (D, 400X) Subcutaneous tumor. (E, 40X) Orthotopic tumor. (F, 400X) Orthotopic tumor. Vessel [red arrow], HCC tumor [black arrows], hepatocyte [green arrow]

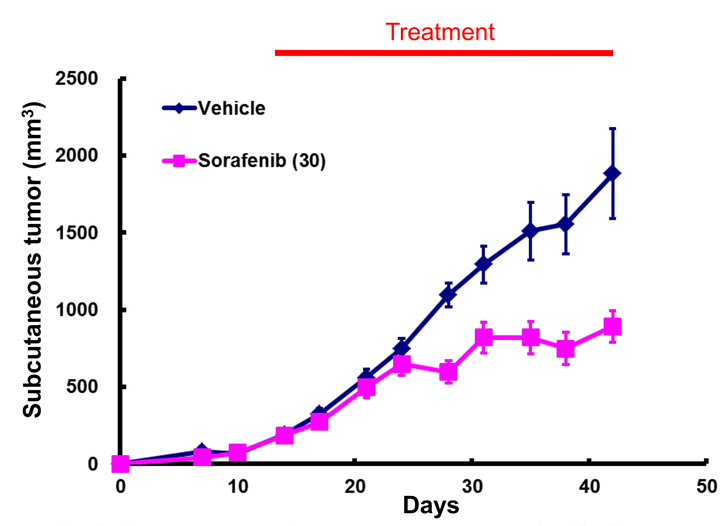

Fig 3. Tumor growth of subcutaneous model (n=7). Our results were demonstrated in mean ± SEM and sorafenib inhibited 59.4% tumor growth . Caliper measurements of tumors were converted into mean tumor volume using the formula: 0.5 × [length × (width)2]. Tumor growth inhibition (%) = (1 – treat / vehicle) *100

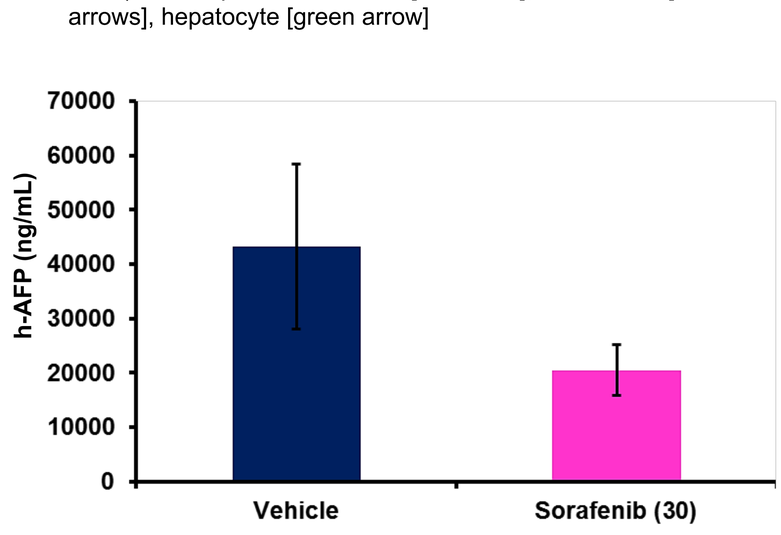

Fig 4. h-AFP of subcutaneous model at 4th week post treatment (n=7). Our results were demonstrated in mean ± SEM and sorafenib inhibited 52.6% h-AFP secretion. H-AFP inhibition (%) = (1 – treat / vehicle) *100. p = 0.13

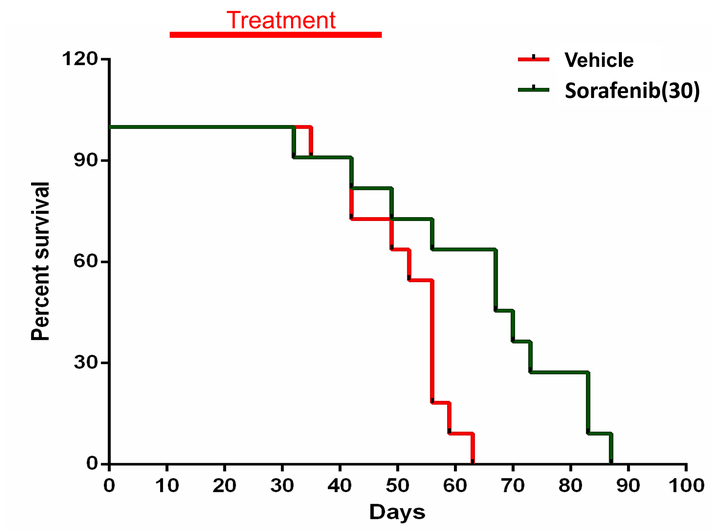

Fig 5. Survival of orthotopic model (n=10). The median survival days were 56 days of vehicle and 67 days of sorafenib. p= 0.008 (death means B.W of mice > 110% , found death or moribund)

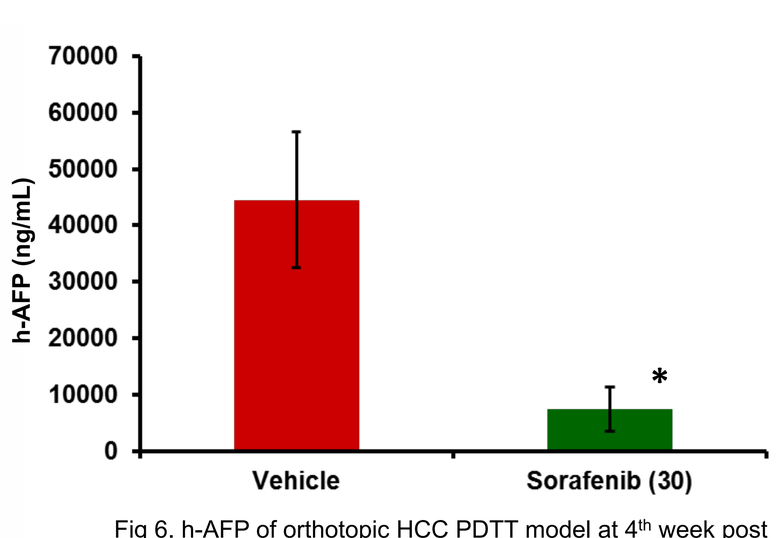

Fig 6. h-AFP of orthotopic HCC PDTT model at 4th week post treatment (n=10). Our results were demonstrated in mean ± SEM and sorafenib inhibited 83.3% h-AFP secretion. h-AFP inhibition (%) = (1 – treat / vehicle) *100. p = 0.013

Conclusion

In this study, we established subcutaneous and orthotopic HCC patient-derived PDX xenograft mouse models that, when combined with the analysis of serum level of the h-AFP biomarker, can serve as a useful platform for evaluating the antitumor activity of drug candidates.

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