A Pro-Regenerative Environment Triggers Premalignant to Malignant Transformation of Senescent Hepatocytes

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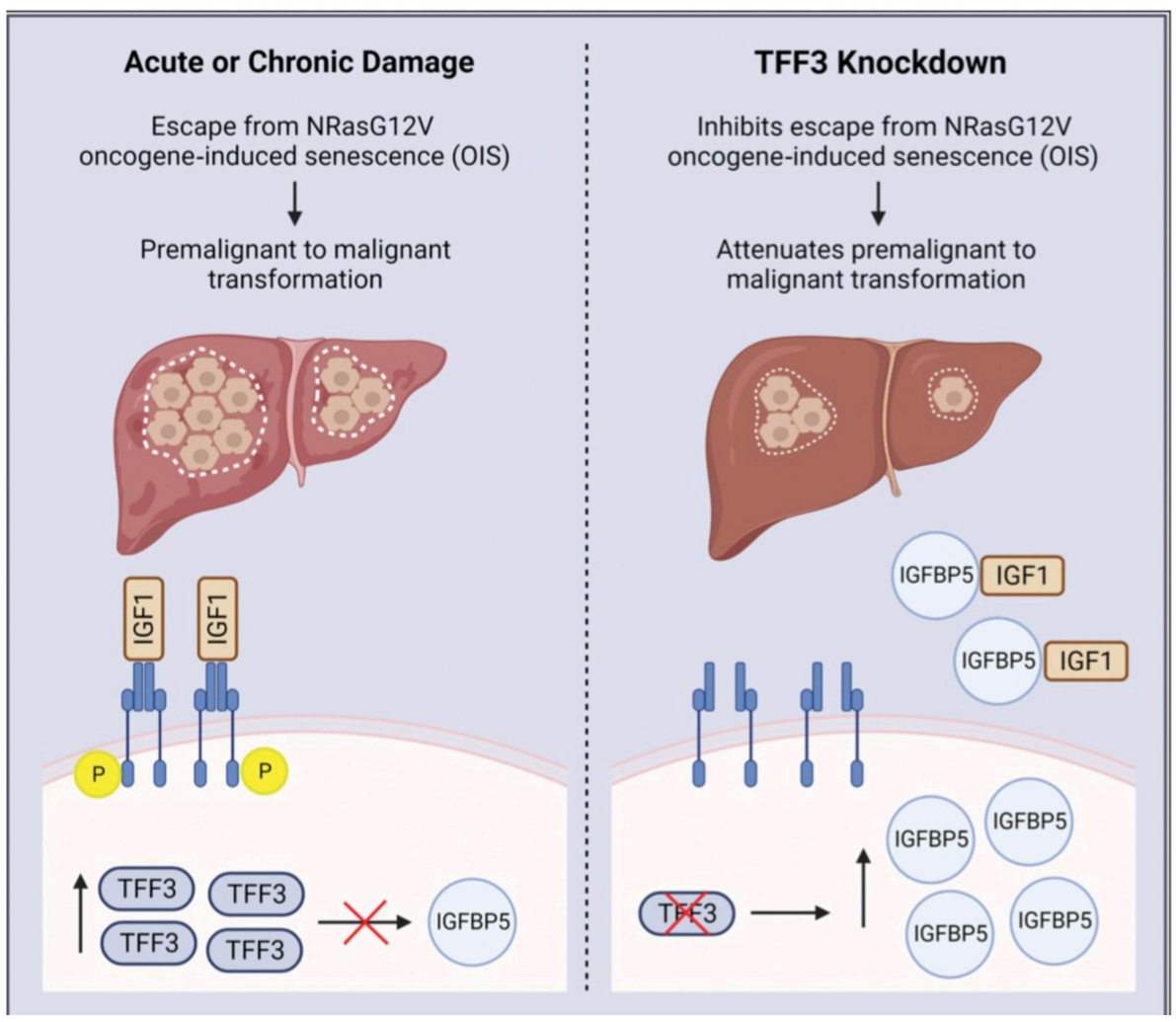
Abstract

The biggest factor improving survival in liver cancer is early detection. Hence, we set up a model to study these early events of liver tumorigenesis and investigated the relationship of premalignant, senescent hepatocytes, a regenerative environment, and the influence of secreted factors. Oncogeneinduced senescence (OIS) was triggered in mouse hepatocytes, which under normal conditions, are eliminated by immunosurveillance. However, by inducing liver damage and a compensatory regenerative response, it was sufficient to trigger immunosurveillance escape of OIS hepatocytes, resulting in premalignant to malignant transformation and hepatocellular tumor development. Trefoil factor 3 (TFF3) was found to be overexpressed in OIS hepatocytes and in hepatocellular carcinoma. TFF3 knockdown was found to strongly attenuate malignant transformation by increasing insulin-like growth factor binding protein 5 (IGFBP5) expression which acts as a tumour suppressor, consequently dampening IGF receptor signaling. Furthermore, analysis of precancerous liver tissue validated TFF3 as an early liver cancer biomarker and its potential for targeted therapies with little toxic side effects.



Material & Methods

An acute/chronic liver damage model was employed to identify potential therapeutic targets, encompassing three critical stages: the induction of stable cellular senescence, the escape from senescence, and the subsequent monitoring of immunosurveillance escape and the progression from a premalignant to malignant state in hepatocytes. This meticulously designed approach captured the trajectory of liver cancer development, and by mirroring the key phases of tumorigenesis, it enabled the identification of novel therapeutic intervention points, offering fresh avenues for targeted treatments.

This experiment employed a multi-faceted approach, integrating human tissue studies, in vivo mouse models, and in vitro cellular assays to comprehensively investigate the mechanisms of liver cancer development and validate therapeutic targets.

- Human samples, including hepatocellular carcinoma (HCC) tissues from various etiologies (HBV, HCV, and NASH) and cDNA from cirrhotic and healthy livers, provided a clinically relevant foundation for identifying and validating molecular targets.
- Mouse models were used to recapitulate liver cancer and chronic liver damage through hydrodynamic tail vein (HDTV) injections for transposon-mediated gene transfer and thioacetamide-induced fibrosis. Tumor formation was studied in immunocompromised mice injected with genetically modified liver cancer cells, while partial hepatectomy allowed for analysis of how acute damage impacts the progression of senescent hepatocytes toward clonal expansion and tumorigenesis.
- In vitro experiments employed diverse liver cancer and hepatocyte cell lines to evaluate gene function, oxidative stress responses, nutrient deprivation effects, and cellular proliferation through shRNA-mediated knockdowns and EdU assays.

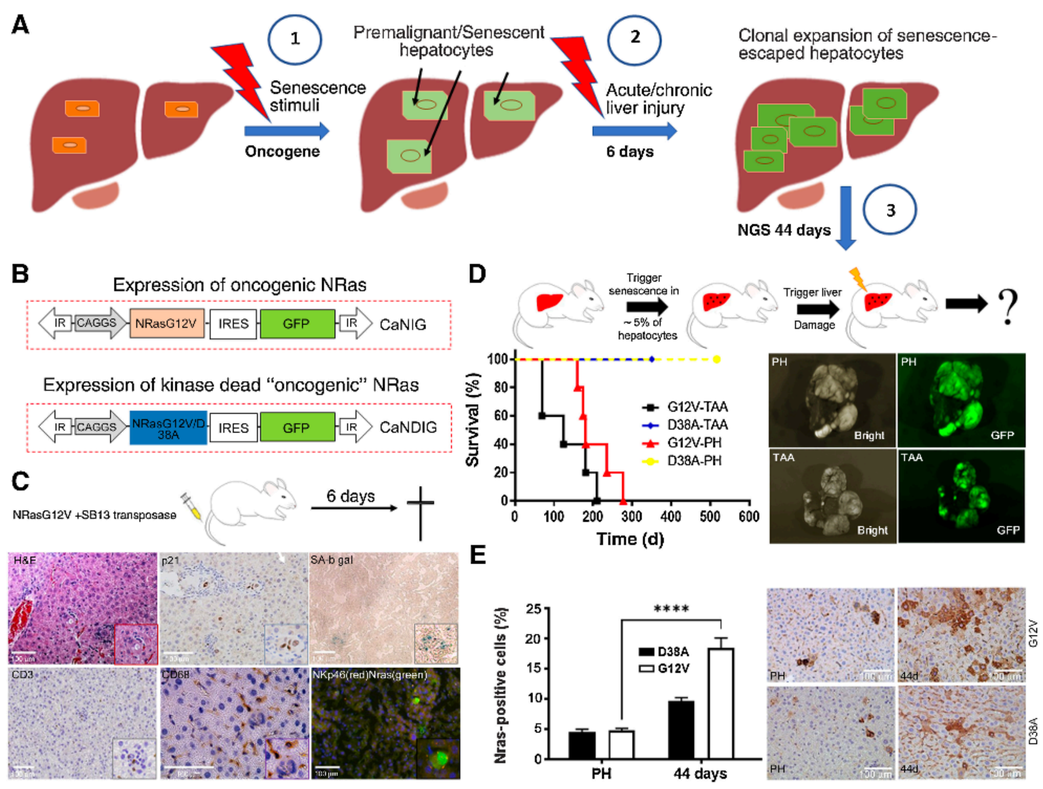


Fig above: In vivo oncogenic-induced senescence escape model.
A, Mouse model to identify early specific molecular targets for liver cancer.
B, Schematic representation of transposable element for stable intrahepatic gene expression of oncogenic NRas (NRasG12V) or an effector loop mutant (NRasG12V/D38A) and the marker gene GFP.
C, OIS (indicated by H&E staining of senescent hepatocyte, by SA-b-gal and p21 staining) and immune surveillance (shown by staining for the T-cell marker CD3, macrophage and monocyte marker CD68, and NK cell marker NKp46) at 6 days post injection.
D, Mice from experimental group (G12V, n ¼ 5) and control group (D38A, n ¼ 4) were either treated with TAA or mice from experimental group (G12V, n ¼ 5) and control group (D38A, n ¼ 6) underwent PH (4). Kaplan–Meier survival curves are shown. Right, macroscopic pictures of the liver with visible tumors as well as GFP scans are shown.
E, Quantification analyses of NRas-positive hepatocytes at 44 days after PH.

Results

1) Oncogene Induced Senescence Is More Likely To Occur In An Immune Deficient State, Mainly Due To Evasion Of Immune-Mediated Clearance
2) Chronic Liver Damage Triggers Senescence Escape, As Cells Undergo Fetal-Like Reprogramming.
This Process Is Reflected By The Levels Of The Biomarker, AFP
3) Knockdown Of TTF3 Delayed Malignant Transformation In Senescent Cells
4) IGFBP5 Is A Key Mediator In Of TTF3 Knockdown, Via Paracrine Effects
IGFBP5 Also Inhibits Activation Of IGFR1, Decreasing Cell Proliferation
5) Knockdown Of IGFBP5 Results In Rapid Expansion Of NRas-Positive Hepatocytes, Reduced Senescent Markers Such As p21 & SA-β-gal & Increased Proliferation Via Ki67
6) Overall, This Showed That Both TFF3 Knockdown & IGFBP5 Upregulation Has Therapeutic Potential In The Treatment Of Liver Cancer

Conclusions & Future works

As a person ages, the senescent cells would accumulate, triggering a microenvironment shift which may promote or suppress tumorigenesis. With TFF3 validated as a biomarker and therapeutic target in not only mouse models but also in patient samples, TFF3 knockdown can be an option for targeted preventative medicine where senescent cells would not promote imflammaging and its associated pro-tumorigenesis effects [1]. This would allow senescent cells to exist as friends within the human body, preserving organ function in old age without driving up the risk of malignant transformation.

Personal Takeaways

With HCC being the third and fourth most common causes of cancer deaths among male and female respectively and only 20% of HCC being detected at a stage where a curative option is feasible, it is timely that such research is being made into finding out new ways to detect potential cancers early [2]. Additionally, such advancements in the understanding of preventative medicine regarding HCC could potentially open doors to understanding other cancers in the human body which were once thought to have a poor prognosis. This could allow new treatments that might not result in such extensive debilitating side effects to be developed as the fight against cancer shifts towards a more precise approach to treating the illness.

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