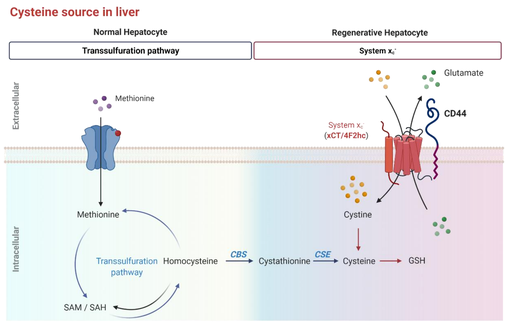
Critical Role of CD44 Ensuring Hepatic Regenerative Capacity as a Modulator for Redox Homeostasis through Stabilizing System Xc



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CD44 plays critical role in liver regeneration through enhanced redox balancing

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1 Introduction

Liver regeneration triggered by fulminant liver damage accompanies proliferation of hepatic progenitor cells. Although there has been considerable interest in the identification of specific markers for hepatic progenitor cells, the physiological role of the those markers during liver regeneration is not fully understood.

2 Aim

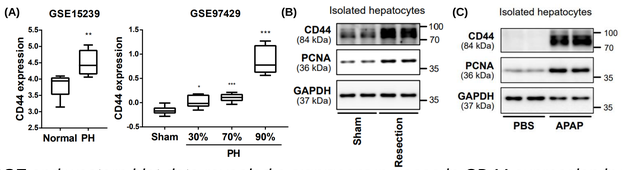
We identified the mechanistic roles of cluster of differentiation (CD)44 on hepatic progenitor cells(HPC), a cell surface marker for hepatic regeneration, in cell proliferation and redox balancing. As CD44 in gastrointestinal cancer cell mediates rewiring of cysteine pool by stabilizing system Xc, we hypothesized that this role is also relevant in the context of HPC which require efficient cysteine metabolism.

3 Method

- **Acetaminophen (APAP)-Induced Liver Injury (AILI)**
  Acetaminophen, 250 mg/kg at single intraperitoneal injection to induce acute injury
- **Partial Hepatectomy (pHx)**
  Resected volume (left lobe) consists approximately 30% of the whole mouse liver
- **Isolation of Primary Hepatocytes**
  Mouse liver perfused with collagenase cultured on dishes coated with collagen and hyaluronic acids, respectively
- **^{13}C Isotope Tracing**
  ^{13}C-cystine used to reveal redox metabolism underlying liver regeneration
- **Hepatocyte-specific knockdown System**
  Using lentiviral albumin promotor, GFP-tagged, CD44 shRNA delivery system

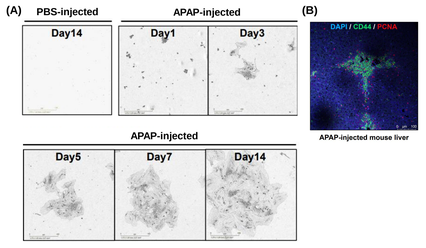
4 Results

1. Expression of CD44 is upregulated in regenerative hepatocytes



GSE and western blot data revealed a significant increase in CD44 expression in liver regeneration undergone (A, B) partial hepatectomy or exposure to excess acetaminophen (C) both in patients and animal models.

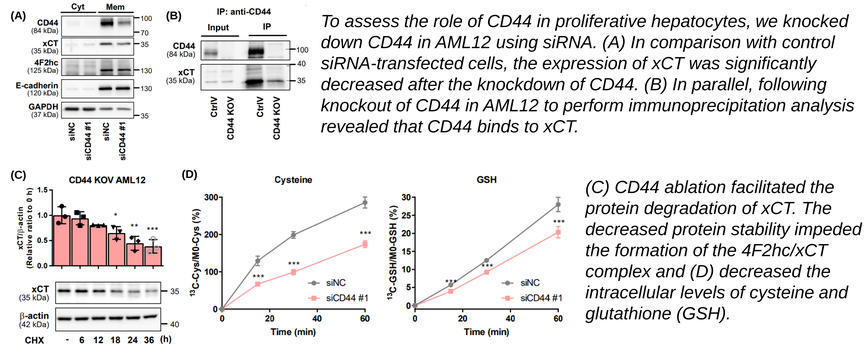
2. CD44-expressing hepatocytes exhibit a proliferative phenotype



Isolated small hepatocytes were cultured on an HA-coated dish for discriminating CD44-expressing hepatocytes.
(A) Only the small hepatocytes isolated from APAP-injected mice proliferated and formed colonies on the HA-coated dish.
(B) Confocal imaging also demonstrates that CD44 was colocalized with PCNA, suggesting CD44 as a suitable HPC marker.

4 Results

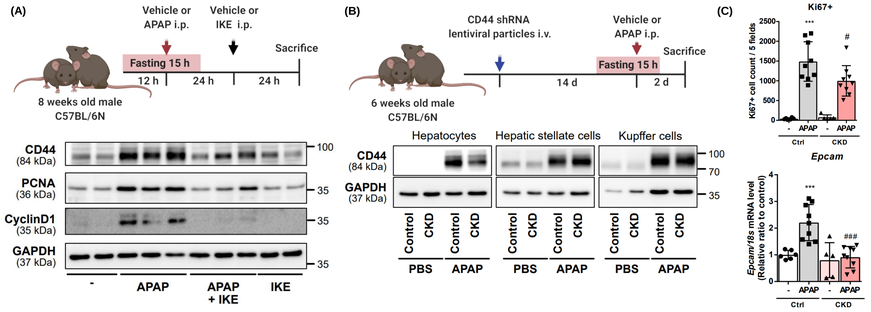
3. CD44 enhances cystine uptake by stabilizing system Xc subunit; xCT



To assess the role of CD44 in proliferative hepatocytes, we knocked down CD44 in AML12 using siRNA. (A) In comparison with control siRNA-transfected cells, the expression of xCT was significantly decreased after the knockdown of CD44. (B) In parallel, following knockout of CD44 in AML12 to perform immunoprecipitation analysis revealed that CD44 binds to xCT.

(C) CD44 ablation facilitated the protein degradation of xCT. The decreased protein stability impeded the formation of the 4F2hc/xCT complex and (D) decreased the intracellular levels of cysteine and glutathione (GSH).

4. Pharmacological and genetical ablation of CD44 resulted in inhibition of liver regeneration



A metabolically stable system Xc- inhibitor, imidazole ketone erastin (IKE) was injected into mice 24 h immediately following APAP injection. (A) IKE administration significantly abrogated the increase of proliferation markers in APAP-injected mice.

(B) Upregulation of CD44 under acute liver injury is exhibited not only in hepatocytes but also in non-parenchymal cells. Therefore, we produced a lentiviral (LV-GFP-Albp-mCD44-shRNA) delivery system and injected this through a mouse tail vein to accomplish hepatocyte-specific CD44 knockdown. (C) Following RT-qPCR revealed that CD44 knockdown reduced xCT expression. Moreover, this treatment inhibited the regenerative process following APAP injection.

5 Conclusions

The obtained results delineate the central role of CD44 in modulating hepatic regenerative capacity. Specifically, CD44 contributes to intracellular redox balancing and cell proliferation by enhancing extracellular cysteine through system Xc.

6 References

1 Kimura K et al. Critical role of CD44 in hepatotoxin-mediated liver injury. Journal of hepatology 2008;48:952-961.
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