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Retinoic acid regulates cell cycle genes and accelerates normal mouse liver regeneration

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ABSTRACT

All-trans retinoic acid (RA) is a potent inducer of regeneration. Because the liver is the principal site for storage and bioactivation of vitamin A, the current study examines the effect of RA in mouse hepatocyte proliferation and liver regeneration. Mice that received a single dose of RA (25 μg/g) by oral gavage developed hepatomegaly with increased number of Ki67-positive cells and induced expression of cell cycle genes in the liver. DNA binding data revealed that RA receptors retinoic acid receptor β (RARβ) and retinoid x receptor α (RXRα) bound to cell cycle genes Cdk1, Cdk2, Cyclin B, Cyclin E, and Cdc25a in mice with and without RA treatment. In addition, RA treatment induced novel binding of RARβ/RXRα to Cdk1, Cdk2, Cyclin D, and Cdk6 genes. All RARβ/RXRα binding sites contained AGGTCA-like motifs. RA treatment also promoted liver regeneration after partial hepatectomy (PH). RA signaling was implicated in normal liver regeneration as the mRNA levels of RARβ, Aldh1a2, Crabp1, and Crbp1 were all induced 1.5 days after PH during the active phase of hepatocyte proliferation. RA treatment prior to PH resulted in early up-regulation of RARβ, Aldh1a2, Crabp1, and Crbp1, which was accompanied by an early induction of cell cycle genes. Western blotting for RARβ, c-myc, Cyclin D, E, and A further supported the early induction of retinoid signal and cell proliferation by RA treatment. Taken together, our data suggest that RA may regulate cell cycle progression and accelerates liver regeneration. Such effect is associated with an early induction of RA signaling, which includes increased expression of the receptor, binding proteins, and processing enzyme for retinoids.

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1. Introduction

All-trans retinoic acid (RA) has long been recognized as a regeneration-inducing derivative of vitamin A. In amphibians, RA causes "super-regeneration" of naturally regenerative organs, while in mammals, RA induces regeneration of organs that do not normally regenerate, such as the adult mammalian lung [1–3]. Interestingly, the liver is the only mammalian organ with highly regenerative properties, making it a prime target for accelerated regrowth through RA. As the principal site for storage and bioactivation of vitamin A in the body, the liver experiences constant RA exposure. Lecithin:retinol acyltransferase-deficient mice, completely lacking in hepatic retinoid stores, have reduced RA levels and impaired liver regeneration in response to partial hepatectomy (PH). Thus, hepatic retinoid storage is required for normal liver regeneration [4].

Despite the essential role of endogenous RA on liver regeneration, there lacks a consensus on the effects of exogenous RA on hepatocyte proliferation *in vivo*, since previous studies have yielded conflicting results [5–8]. Early *in vitro* studies reported that RA is a potent inhibitor of DNA synthesis in primary rat hepatocytes [9]. Similarly, when administered after PH, RA disrupted rat hepatocyte proliferation *in vivo* by repressing early response genes and increasing the activity of transglutaminase and ornithinedecarboxylase [10–14]. In contrast to these findings, other studies have shown that RA enhances hepatocyte

Abbreviations: Aldh1a2, aldehyde dehydrogenase family 1 member A2; Cdk1, Cyclin-dependent kinase 1, Cdc25, cell division cycle 25; ChIP-seq, chromatin immunoprecipitation followed by next generation sequencing; Crabp1, cellular retinoic acid binding protein 1; Crbp1, cellular retinol binding protein 1; E2f, E2f transcription factor; Foxm1, forkhead box M1; KEGG, Kyoto encyclopedia of Genes; Pcna, proliferating cell nuclear antigen; PH, partial hepatectomy; qRT-PCR, real-time quantitative reverse transcription PCR; RA, all-trans retinoic acid; RAR, retinoic acid receptor; RXR, retinoid X receptor; WT, wild type ;.

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proliferation and survival. RA augmented TNFα-stimulated mouse hepatocyte DNA synthesis *in vitro* [15]. The direct mitogenic effect of RA has also been demonstrated in normal hepatocyte proliferation of both mice and rats [16,17]. Post-operative administration of RA or synthetic retinoid NIK33 enhanced hepatocyte proliferation in the regenerating rat liver [18].

It is problematic that the existing literature has such contradictory findings since RA is considered a chemotherapeutic agent against certain types of cancer. Despite its anti-proliferative and differentiation effects against malignant cells, it is necessary to acknowledge the role of RA in proliferation and regeneration as well as the clinical implications. Although RA is often used to treat patients with acute promyelocytic leukemia (APL), major complications can manifest in the form of hepatomegaly and hepatotoxicity [19]. The conflicting results from previous studies may be attributable to differences in dosage, route of administration, and animal species. Another important factor to consider is the timing of exogenous RA administration. In the previous studies, RA was administered after surgical resection of the liver [10–14,18]. The hepatoprotective role of RA preconditioning has recently been proposed in an ischemic/reperfusion injury model [20], but there is no evidence in the literature that clearly documents the effects of RA treatment prior to PH-induced liver regeneration. Therefore, this study aims to clarify the role of RA in the regenerating liver by demonstrating, for the first time, the effects of RA administration prior to PH.

In this study, RA pretreatment is shown to accelerate PH-induced liver regeneration in mice by increasing the expression of genes encoding Cyclin-Cdk complexes. Moreover, the data indicate that RA pretreatment promoted hepatocyte proliferation in the regenerating mouse liver by modulating cell cycle progression through direct binding by retinoic acid receptor β (RARβ) and retinoid x receptor α (RXRα). Together, these findings emphasize the potential utility of employing retinoids to facilitate liver regrowth following injury.

2. Materials and methods

2.1. Mice, partial hepatectomy, and sample preparation

Wild type (WT) male mice (3–5 months old) were housed in steel micro-isolator cages (4 mice per cage) at 22 °C with a 12-h/12-h light/dark cycle. Food and water were provided *ad libitum* throughout the entire study. RA (25 μg/g) (Sigma–Aldrich Corp., St. Louis, MO) or vehicle control (carboxymethyl cellulose) (Sigma–Aldrich Corp., St. Louis, MO) was administered by oral gavage 48 h prior to surgery. Standard two-thirds liver resection was performed using the procedure previously described [21–23]. Sham-operated mice were included as controls. Surgeries were performed between 9:00 and 11:00 AM. Mice were killed at indicated time points and their liver and body weight recorded at the time of death were used to calculate liver-to-body weight ratio. The data presented were calculated from the mean of three to five mice per time point. Liver tissues were collected and snap frozen in liquid nitrogen and stored at −80 °C. A section of each liver was fixed in 10% formalin, embedded in paraffin, and stained with hematoxylin and eosin for histological analysis. All animal experiments were conducted in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals and the Institutional Animal Care and Use Committee (IACUC) at the University of California, Davis.

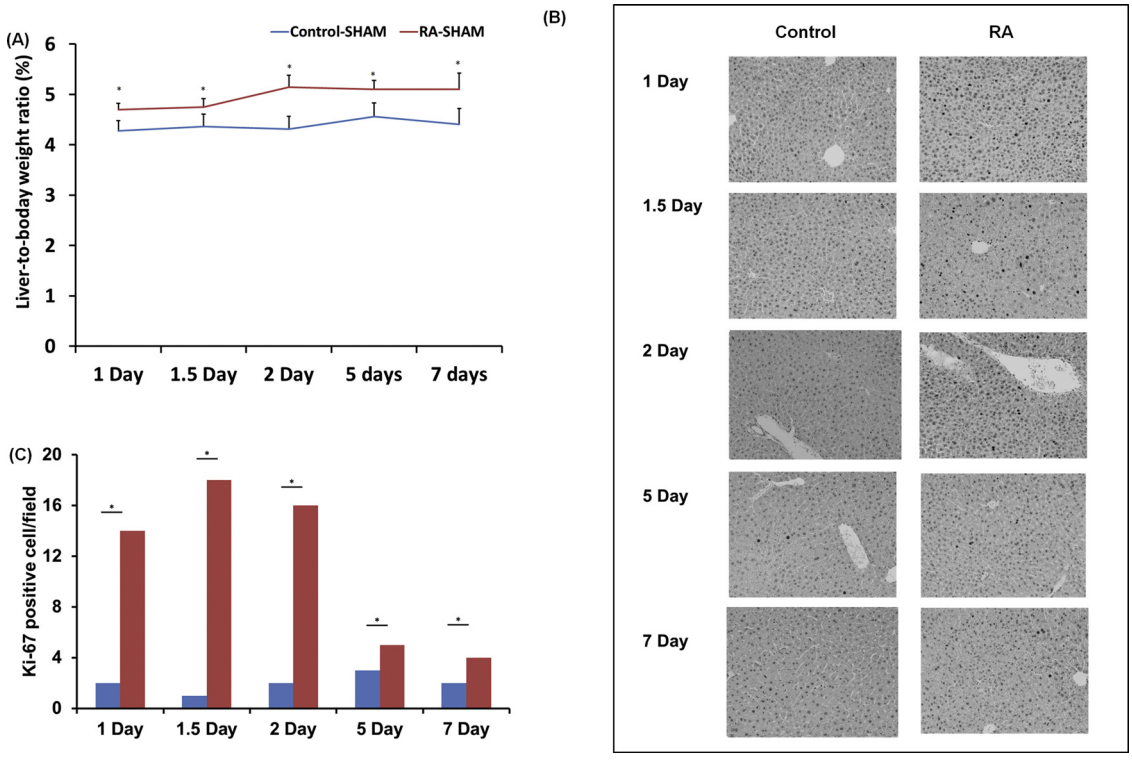

Fig. 1. Increased hepatocyte proliferation in RA-treated mouse liver. WT mice were treated with RA or vehicle by oral gavage 48 h prior to a sham operation. (A) Mice were sacrificed and weighed 1, 1.5, 2, 5 and 7 days after surgery to calculate differences in liver to body weight ratio between the groups. (B) Representative photomicrographs of Ki67 immunohistochemical staining of liver sections from WT mice with and without RA treatment at indicated time points ($n \geq 4$). (C) Ki67-positive cells in the livers of WT mice with and without RA pre-treatment after sham operation. The number of proliferating hepatocytes was determined by counting Ki67-positive hepatocytes in at least 15 microscopic fields (20X) per liver sample. Liver sections from all mice were used for analyses. Means ± SD are graphed with * indicating $p < 0.05$.