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The epidermal growth factor receptor ligand amphiregulin is a negative regulator of hepatic acute-phase gene expression☆

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***Background/Aims*: The modulation of the hepatic acute-phase reaction (APR) that occurs during inflammation and liver regeneration is important for allowing normal hepatocellular proliferation and the restoration of homeostasis. Activation of acute-phase protein (APP) gene expression by interleukin-6 (IL-6)-type cytokines is thought to be counteracted by growth factors released during hepatic inflammation and regeneration. The epidermal growth factor receptor (EGFR) ligand amphiregulin (AR) is readily induced by inflammatory signals and plays a nonredundant protective role during liver injury. In this paper, we investigated the role of AR as a modulator of liver APP gene expression.**

***Methods*: Expression of APP genes was measured in the livers of $AR^{+/+}$ and $AR^{-/-}$mice during inflammation and regeneration and in cultured liver cells treated with AR and oncostatin M (OSM). Crosstalk between AR and OSM signalling was studied.**

***Results*: APP genes were overexpressed in the livers of $AR^{-/-}$ mice during inflammation and hepatocellular regeneration. In cultured AR-null hepatocytes and human hepatocellular carcinoma (HCC) cells after AR knockdown, APP gene expression is enhanced. AR counteracts OSM-triggered signal transducer and activator of transcription 3 signalling in hepatocytes and attenuates APP gene transcription.**

***Conclusions*: Our data support the relevance of EGFR-mediated signalling in the modulation of cytokine-activated pathways. We have identified AR as a key regulator of hepatic APP gene expression during inflammation and liver regeneration.**

***Keywords*: Acute-phase proteins; Amphiregulin; Oncostatin; Liver regeneration**

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Abbreviations: APR, acute-phase reaction; APP, acute-phase protein; IL, interleukin; EGFR, epidermal growth factor receptor; AR, amphiregulin; OSM, oncostatin M; STAT, signal transducer and activator of transcription; TNF, tumour necrosis factor; JAK, Janus-activated kinase; TGF, transforming growth factor; HB-EGF, heparin-binding EGF-like growth factor; BTC, betacellulin; EREG, epiregulin; LPS, lipopolysaccharide; MEK1, extracellular regulated kinase kinase 1; ActD, actinomycin D; α1-ACT, α1-antichymotrypsin; SAA, serum amyloid A; AGP2, α1-acid glycoprotein 2; ERK, extracellular regulated kinase; SHP2, Src homology 2 domain-containing tyrosine phosphatase; SOCS, suppressor of cytokine signalling; siRNA, short interfering RNA; ChIP, chromatin immunoprecipitation; PH, partial hepatectomy.

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1. Introduction

Upon tissue injury, infection, and inflammation, a potent systemic reaction known as the *acute-phase response* (APR) is triggered to maintain homeostasis and to remove the causative agent. In this process, the liver undergoes significant functional adaptations, including alterations in metabolic pathways and profound changes in the expression of plasma proteins produced by hepatocytes [1,2]. These proteins are collectively known as *acute-phase proteins* (APPs), and although some of them decrease during the APR, the majority are significantly induced [2,3]. The APPs serve a number of important roles in the restoration of homeostasis, including microbicidal and phagocytic functions, haemostatic functions, antithrombotic effects, modulation of cholesterol metabolism, and antiproteolytic effects [1–3]. Changes in hepatic APP expression are predominantly mediated by the concerted action of inflammatory cytokines such as interleukin (IL)-1β, tumour necrosis factor-α (TNF-α), IL-6, and IL-6-type cytokines like oncostatin M (OSM), mainly produced by inflammatory cells [1,3,4]. Upon binding to their cognate receptors, these cytokines trigger intracellular signalling pathways mediating the upregulation of APPs by three important transcription factors: nuclear factor-κB, CCAAT/enhancer binding protein, and signal transducers and activators of transcription (STAT) [3–6]. IL-6-related cytokines share a common receptor subunit, gp130, that heterodimerises with each cytokine-specific receptor subunit upon cytokine binding, resulting in the rapid tyrosine phosphorylation and activation of Janus-associated kinases (JAK), which in turn phosphorylate and activate the STATs [7–9]. Binding of STAT3 to STAT recognition sites in the promoter of APP genes is essential for their transcriptional induction in hepatocytes [9–11].

Equally important to the elucidation of the mechanisms involved in the upregulation of APPs is the understanding of the processes that limit the extent of the reaction. Chronic changes in the levels of APPs are considered detrimental to the organism, and the identification of such attenuating mechanisms may help to control the hepatic inflammatory response [1]. The APR also occurs during liver injury and regeneration. It was observed early on that the magnitude of the APR elicited after partial hepatectomy (PH) in rodents was significantly reduced when compared to the APR triggered by acute inflammation, indicating that in liver regeneration, the extent of the APR was somehow limited [12,13]. Similar observations were made in patients undergoing major hepatectomy [14]. Interestingly, when an APR is elicited by the administration of proinflammatory compounds, such as bacterial lipopolysaccharide, liver regeneration in response to PH is significantly compromised [15,16]. These findings suggested that modulation of the APR is important for normal liver regeneration to proceed and that growth factors released during liver injury and inflammation could mediate the suppression of the APR in the regenerating liver [17–19]. In vitro experiments using cultured mouse and human liver cells subsequently demonstrated that IL-6-stimulated APP gene expression was attenuated in the presence of epidermal growth factor (EGF) [20,21]. This effect was mediated through activation of downstream signalling from the EGF receptor (EGFR), a transmembrane tyrosine kinase [21]. In vivo, the EGFR can be activated by a broad family of ligands that, in addition to EGF, includes transforming growth factor-α (TGF-α), heparin-binding EGF-like growth factor (HB-EGF), betacellulin (BTC), epiregulin (EREG), and amphiregulin (AR) [22]. The expression of some of these growth factors, such as TGF-α, HB-EGF, and AR, is upregulated during liver regeneration [22]. Although functional redundancy may exist among them, studies performed in knockout mice indicated that AR could play a prominent role in this process [23]. In this paper, we provide evidence demonstrating that AR is also an important determinant in the regulation of the hepatic APR, controlling the extent of APP expression in hepatocytes.

2. Materials and methods

2.1. In vivo experiments

Experiments were performed according to our institution's guidelines for animal experimentation. $AR^{+/+}$ and $AR^{-/-}$ mice have been described [23,24]. Acute inflammation was induced in male $AR^{+/+}$ and $AR^{-/-}$ littermate mice (20 g; four to five mice per condition) by a single intraperitoneal (IP) injection of *Escherichia coli* (serotype 0111:B4) lipopolysaccharide (LPS) from Sigma (St. Louis, MO, USA; 15 mg/kg). PH was performed as reported [23]. Mice were sacrificed by cervical dislocation, and liver samples were snap-frozen in liquid N_2 for subsequent analyses.

2.2. Cell culture and treatments

Mouse hepatocytes were isolated by collagenase perfusion and cultured as described [24]. Treatments were performed the day after hepatocyte isolation in the absence of serum. The human hepatocellular carcinoma (HCC) cell lines HepG2 and PLC/PRF/5 as well as the murine hepatocyte AML12 cells were from the ATCC and were grown as reported [25,26]. Cells were treated with human and mouse recombinant OSM (R&D Systems, Minneapolis, MN, USA), human AR (Sigma), the extracellular regulated kinase kinase 1 (MEK1) inhibitor UO126 (Promega, Madison, WI, USA), the JAK2 inhibitor AG490 (Calbiochem, San Diego, CA, USA), and actinomycin D (ActD; Sigma).

2.3. RNA isolation and analysis of gene expression

Total RNA was extracted, and reverse transcription and quantitative real-time polymerase chain reaction (PCR) were performed as described [24]. Primers used for the analysis of AR, HB-EGF, TGF-α, EGF, EREG, BTC, and suppressor of cytokine signalling 3 (SOCS3) gene expression have been reported [16,24]. The following primers were also used:

- Sense 5′-AGGTGGTCCATAAGGCTGTG-3′ and antisense 5′-CTTGCTTGGGATTGGTGACT-3′ for human α1-antichymotrypsin (α1-ACT).