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Oncostatin M induces an acute phase response but does not modulate the growth or maturation-status of liver progenitor (oval) cells in culture

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Abstract

Following acute injury, the liver regenerates through hepatocyte division. If this pathway is impaired, liver repair depends on the recruitment of adult liver progenitor (oval) cells. Mice fed a choline deficient, ethionine supplemented (CDE) diet possess substantial numbers of oval cells, which can be isolated, or examined in vivo. Oncostatin M (OSM) has been shown to induce maturation of murine fetal hepatoblasts into hepatocytes. We recently confirmed this in human fetal liver cultures. Here, we show that liver OSM expression increases in mice fed a CDE diet and CDE-derived oval cell isolates express OSM and its receptor (OSMR). Oval cell lines (PIL cells), as well as primary oval cell cultures, displayed STAT-3 phosphorylation following OSM stimulation. OSM had no effect on the growth of primary oval cells, but it was pro-apoptotic to PIL cells, suggesting that the two cell models are not directly comparable. Expression of PCNA and cyclin D1 was not affected by OSM treatment. No evidence was obtained to suggest an effect on oval cell maturation with OSM treatment. However, decreased albumin production, accompanied by increased expression of haptoglobin and fibrinogen, suggests that OSM induced an acute phase reaction in cultured oval cells.

Keywords: Oncostatin M; Progenitor cells; Mouse; Proliferation; Differentiation; Maturation; Apoptosis; Liver regeneration

Introduction

The adult mammalian liver performs several critical functions, including metabolic homeostasis and xenobiotic clearance. As a vital organ, its function needs to be efficiently restored following damage. Following acute injury (caused, for example, by tissue trauma or paracetamol poisoning), liver regeneration is accomplished through the division of remaining healthy hepatocytes. However, if this pathway is impaired, then regeneration relies primarily on the recruitment of adult liver progenitor cells, which replenish damaged hepatic cells through a coordinated process of proliferation, migration and differentiation, to produce functional hepatocytes or cholangiocytes. This process is invoked following chronic or carcinogenic liver injury, such as that typically seen in patients suffering prolonged hepatitis B or C infection, liver iron overload or alcohol poisoning [1–3]. Progenitor cell recruitment is also well documented in rodents treated with carcinogens such as Azo dyes [4] or ethionine [5,6], or with genetic abnormalities such as

Abbreviations: LT, Lymphotoxin; OSM, Oncostatin; LIF, Leukaemia Inhibitory Factor; CDE, Choline deficient, ethionine supplemented; IL, Interleukin; TNF, Tumour necrosis factor; AFP, Alpha fetoprotein; TAT, Tyrosine aminotransferase; GGT IV, Gamma glutamyl transpeptidase IV; CK, Cytokeratin; PCNA, Proliferating Cell Nuclear Antigen.

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mutation of the murine Wilson's disease gene, leading to copper accumulation in the liver [7]. Interestingly, several investigators have reported a correlation between the extent of progenitor cell proliferation in the liver and the likelihood of disease progression to liver cancer [1,5,8]. This, together with evidence documenting the ease of transformation of liver progenitor cells in culture [9,10], suggests that oval cells may represent a cellular precursor to hepatocellular carcinoma.

The first formal description of liver progenitor cells (made in 1956) suggested they be termed "oval" cells due to their distinct ovoid shape in cross-section [11]. In the ensuing years, this term has been used widely to describe phenotypically similar cell populations observed in liver specimens of mouse, rat, hamster, duck and human origin (for review, see [12–14]). They possess a mixed phenotype, sharing characteristics typical of both fetal and adult hepatocytes, as well as biliary epithelial cells. A variety of cytokines have been implicated in the regulation of oval cell proliferation in vivo and in vitro. These include interleukin (IL)-6, Leukemia Inhibitory Factor (LIF), interferon-γ, lymphotoxin-β and tumour necrosis factor (TNF) [8,15–19]. LIF and IL-6 are members of a family of structurally-related cytokines, which also includes IL-11, ciliary neurotrophic factor and Oncostatin M (OSM). Recent findings have identified OSM as an important mediator of both fetal and adult liver homeostasis. OSM coordinates the late stages of embryonic liver development, promoting the maturation of fetal hepatoblasts into hepatocytes and terminating liver hemopoiesis [20–22]. In the adult, OSM mediates the production of acute phase proteins and promotes the proliferation of hepatocytes following acute liver injury invoked by hepatic resection [23]. As oval cells have characteristics in common with both fetal and adult hepatocytes, we hypothesised that OSM may also affect their biology in vivo or in vitro. Thus, we undertook the present study to clarify whether OSM signalling is invoked during oval cell proliferation in whole mouse liver and to clarify the effect, if any, of OSM signalling on liver progenitor oval cells in culture.

Materials and methods

Animals

Four week old, male mice were used in these studies. Wild type mice were of the C57BL/6J inbred strain. TNF receptor knockout mice were as described previously [24]. Mice were fed a choline deficient, ethionine supplemented (CDE) diet, as described previously [25]. Following 0, 7, 14, 21 or 28 days on the diet, animals were sacrificed and liver portions removed for immunohistochemical and RNA analysis or for cell isolation.

Isolation of cells from whole liver for RT-PCR analysis

To determine the expression pattern of OSM and its receptor in isolated cell types, hepatocytes, oval cells and inflammatory cells were isolated as previously described [8]. Briefly, mice were fed either a control or CDE diet for 2 weeks. Livers were digested by collagenase perfusion and the resulting cell suspension subjected to centrifugal elutriation to separate cells according to size. Elutriation fraction 3 (which contains oval cells) was then purified further using the MiniMACS™ system, with anti-CD45 beads (Miltenyl Biotech). The oval cell fraction (CD45 negative) was determined to be >99% pure oval cells by immunohistochemistry using A6 (an oval-cell directed antibody [26]). The CD45 positive inflammatory cell fraction was found to be >98% pure based on immunohistochemistry for CD45.

Cell lines

The p53 immortalised liver (PIL) progenitor cell lines, PIL-2 and PIL-4, were used in this study as a model of cultured oval cells. Our laboratory has demonstrated that primary oval cell cultures and the PIL cell lines are highly similar in their morphology and pattern of gene expression [10,19,27]. PIL cells were maintained in supplemented William's E medium with 5% fetal bovine serum (FBS; Invitrogen, USA), as described previously, except the final concentration of dexamethasone used was 10^{-6} M rather than 10^{-7} M [10]. NIH3T3 fibroblasts (used as a positive control in Western blot analyses) were maintained in Dulbecco's minimal essential medium with 10% fetal bovine serum.

Differentiation of PIL cell lines

To stimulate their differentiation, PIL-2 or PIL-4 cells were seeded at a final density of 0.25×10^6/mL in either Matrigel® (BD Biosciences, USA), as recommended by the manufacturer, or in RPMI supplemented with ITS (BD Biosciences, USA), epidermal growth factor (50 ng/mL), nicotinamide (10 mM) and dexamethasone (10^{-7} M). Following 10 days, cells were harvested and RNA isolated for gene expression analysis.

Isolation and culture of primary oval cells

Oval cells were isolated from 2 week CDE-fed mouse liver using a modification of a protocol described previously [28]. Briefly, livers were perfused using a two-step EGTA/collagenase method to dissociate cells. The resulting cell suspension was then pelleted and transferred to a trypsinising flask containing 12,000 U type VIII collagenase (Sigma, USA), 20 mg/mL pronase (Sigma, USA), 5% trypsin/EDTA (Invitrogen, USA) and 0.2% DNase I (Sigma, USA) in PBS for 1 h at 37°C. The digested cell fraction was then filtered