The effect of age and calorie restriction on HIF-1-responsive genes in aged liver

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Abstract

Hypoxia inducible factor-1 (HIF-1) regulates transactivation of several genes in response to hypoxia condition. We explore hepatic HIF-1 responsive gene regulation during aging and the age-related changes of the HIF-1 related gene activation in young and old rats. Results indicate that the aging process induces the activation of HIF-1α, which is accompanied by increased HIF-1 DNA binding. This increased binding activity is accompanied by the increase of HIF-1-dependent genes, heme oxygenase-1 (HO-1), vascular endothelial growth factor (VEGF), erythropoietin (EPO), and inducible nitric oxide synthase (iNOS), which all showed remarkable up-regulation during aging process. In contrast, the increased HIF-1 related gene expression was effectively blunted by the anti-oxidative action of calorie restriction in aged rat liver.

We propose that age-related HIF-1 binding activity may well be influenced by the increased pro-oxidative conditions of aged animals, which up-regulate HIF-1-dependent gene expression.

Abbreviations: EPO – erythropoietin; HIF-1 – hypoxia inducible factor 1; HO-1 – heme oxygenase-1; iNOS – inducible nitric oxide synthase; NO – nitric oxide; Nox – nitrate plus nitrite; ROS – reactive oxygen species; VEGF, vascular endothelial growth factor

Introduction

Hypoxia inducible factor-1 (HIF-1) regulates the transcription of various genes that are known to affect cell proliferation (Carmeliet et al. 1998), angiogenesis (Lee et al. 2003), and inflammation (Cramer et al. 2003). Among many other genes activated under hypoxic conditions are erythropoietin (EPO) (Semenza and Wang 1992), cytokines, vascular endothelial growth factor (VEGF) (Levy et al. 1995), endothelin-1 (ET-1) (Sinor and Greenberg 2000), platelet-derived growth factor-β (PDGF-β) (Jung et al. 2000), and basic fibroblast growth factor (bFGF) (Niwa et al. 1999), and glucose transporter, Glut 1 (Tomes et al. 2003).

In addition, hypoxia also modulates the expression of genes associated with inducible nitric oxide (NO) synthase (iNOS) (Palmer et al. 1998; Niwa et al. 1999; Jung et al. 2000) and heme oxygenase-1 (HO-1) (Lee et al. 1997). Furthermore, in hypoxic condition, NO regulates the expression of HIF-1α (Agani et al. 2002), that is constitutively expressed. Under normoxic conditions, activation of HIF-1 modulates array of expression of genes whose products are important for tumorigenesis, survival and tumor progression.

Evidences strongly indicate that most of age-related changes and damages are casually related to oxidative stress with increased reactive oxygen...
species (ROS) as consequences of aerobic metabolism (Yu 1994; Martin et al. 1996). Thus, documentation of changes in redox sensitive transcription factor, HIF is important as HIF modulates VEGF and EPO gene expression that play a major role in vascular maintenance and angiogenesis.

At present, the status of HIF and its modulation on the HIF-dependent gene expression during aging process are not well known. Much evidence strongly indicates that most of age-related changes and damages are causally related to oxidative stress with increased ROS and reactive nitrogen species (RNS) as a consequence of aerobic metabolism (Yu 1994; Martin et al. 1996). Age-related molecular damage and the functional loss due to redox imbalance are accompanied with the molecular events involved in inflammatory reaction (Chung et al. 2002). It has been reported that inflammatory cytokines are likely be under the influence of HIF-1α or NF-κB transcriptional regulation of the hypoxic condition on mast cells (Jeong et al. 2003).

Recent reports well documented that calorie restriction (CR) can suppress the age-related redox imbalance, increased cyclooxygenase (COX) activity, and pro-inflammatory prostaglandins (PG) (Chung et al. 1999; Kim et al. 2000). These beneficial CR effects were further exhibited at the level of gene regulation (Yu 2000) as shown by the suppression of the age-related increase of COX-2 mRNA and protein levels via the modulation of NF-κB and inhibitory κB (IκB) (Kim et al. 2000).

The present study was carried out to explore the modulation mode of the age-related transactivation of HIF-1 activity and changes of HIF-1-dependent genes such as VEGF, EPO, iNOS, and HO-1 in the hope of a better understanding of their possible interaction in relation to the signal aging process.

**Materials and methods**

**Animals**

Rat maintenance procedures for specific-pathogen free (SPF) status and dietary composition of chow have been previously reported (Yu 2000). Briefly, male, SPF Fischer 344 rats were fed a diet of the following composition: 21% soybean protein, 15% sucrose, 43.65% dextrin, 10% corn oil, 0.15% a-methionine, 0.2% choline chloride, 5% salt mix, 2% vitamin mix, and 3% Solka-Floc. Rats at 6, 12, 18, and 24 months of age were sacrificed by decapitation and the livers were quickly removed and rinsed in ice-cold buffer [100 mM Tris, 1 mM EDTA, 0.2 mM phenylmethylsulfonyl fluoride (PMSF), 1 μM pepstatin, 2 μM leupeptin, 80 mg trypsin inhibitor per litre, and 10 μM N-CBZ-LEU-LEU-LEU-AL (CBZ-LLL, proteasome inhibitor), pH 7.4]. The tissue was immediately frozen in liquid nitrogen and stored at −80 °C. Histopathological examination revealed no evidence of nephrotic lesions detected in these soy-protein fed rats even at 24 months of age (Iwasaki et al. 1988).

**Materials**

All chemical reagents were obtained from Sigma Chemical Company (St. Louis, MO, USA), except as noted. The radionucleotide [γ-32P]-ATP (250 Ci), and Western blotting detection reagents, horseradish peroxidase-conjugated donkey anti-rabbit antibody and anti-mouse antibodies were obtained from Amersham Pharmacia Biotech (Bucks, UK). RNAzol™ B was obtained from TEL-TEST, Inc. (Friendwood, TX, USA). Primers of HIF-1α, VEGF, and EPO for RT-PCR were synthesized by BIOBASIC (Ontario, Canada). Primers of iNOS, HO-1, and GAPDH for RT-PCR were synthesized by Bioneer (Daejeon, Korea). Reverse transcriptase was from Gibco-Bethesda Research Laboratory (MD, USA), Taq DNA polymerase and 10 × PCR buffer were from Perkin Elmer (CA, USA). Anti-HIF-1α, anti-HIF-1β antibodies were obtained from Novus Biologicals, Inc. (Littleton, CO, USA). Anti-iNOS, anti-VEGF, and anti-EPO antibodies were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA). Anti-HO-1 antibody was obtained from Stressgen (Victoria, BC, Canada). Horseradish peroxidase-conjugated donkey anti-sheep/goat IgG was purchased from Serotec (Oxford, UK). Polyvinylidene difluoride (PVDF) membranes were obtained from Millipore Corporation (Bedford, MA, USA). All other materials were obtained in the highest available grade.