Toxicology Letters 240 (2016) 81–92

Contents lists available at ScienceDirect

Toxicology Letters

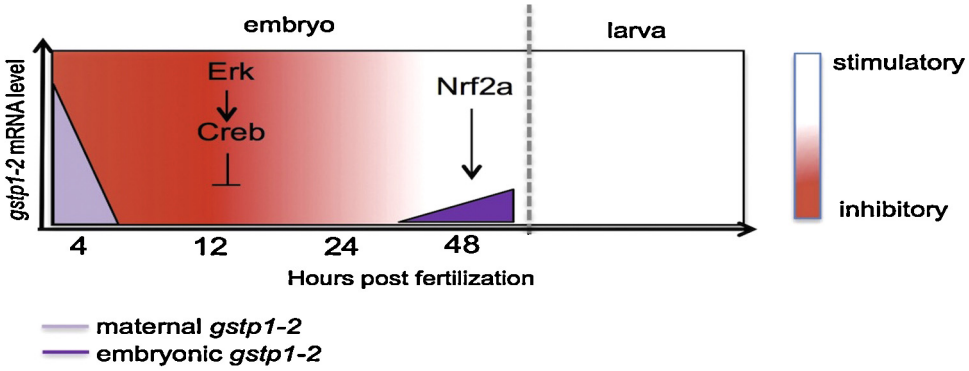
journal homepage: www.elsevier.com/locate/toxlet

Erk-Creb pathway suppresses glutathione-*S*-transferase pi expression under basal and oxidative stress conditions in zebrafish embryos

Jelena Hrubik, Branka Glisic, Svetlana Fa, Kristina Pogrmic-Majkic, Nebojsa Andric*

Laboratory for Ecotoxicology, Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, Novi Sad, Serbia

GRAPHICAL ABSTRACT



HIGHLIGHTS

- Erk supresses basal Gst Pi expression in zebrafish embryos.
- Erk does not change the tBHQ-induced *gstp* mRNA in zebrafish embryos.
- Increase in Erk activity suppresses *gstp* mRNA in the PMA-exposed zebrafish embryos.
- Creb acts as a proximal transmitter of the Erk effect on *gstp* mRNA in zebrafish embryos.

ARTICLE INFO

Article history:
Received 7 July 2015
Received in revised form 14 October 2015

ABSTRACT

Transcriptional activation of phase II enzymes including glutathione-*S*-transferase pi class (Gst Pi) is important for redox regulation and defense from xenobiotics. The role of extracellular signal-regulated kinase (Erk) and protein kinase B (Akt) in regulation of Gst Pi expression has been described using adult mammalian cells. Whether these signaling pathways contribute to Gst Pi expression during

Abbreviations: Ahr, aryl hydrocarbon receptor; Akt, protein kinase B; AOE, antioxidant enzyme; AP-1, activator protein 1; cAMP, cyclic adenosine monophosphate; *cat*, catalase; CRE, cAMP response element; Creb, cAMP response element-binding protein; *cyp1a*, cytochrome P450 1a; *cyp3a65*, cytochrome P450 3a65; *ef1α*, elongation factor 1α; Erk, extracellular signal-regulated kinase 1/2; Gapdh, glyceraldehyde 3-phosphate dehydrogenase; *gpx1*, glutathione peroxidase 1; GSH, glutathione, gamma-glutamyl-cysteinyl-glycine; Gst, glutathione-*S*-transferase; *hmox1*, heme oxygenase (decycling) 1; hpf, hours post fertilization; MAPKs, mitogen-activated protein kinases; Mek, mitogen-activated protein kinase kinase; NF-κB, nuclear factor kappa-light-chain-enhancer of activated B cells; Nrf2, nuclear factor (erythroid-derived 2)-like 2; Keap1, Kelch-like ECH-associated protein 1; P450s, cytochrome P450 enzymes; PAHs, polycyclic aromatic hydrocarbons; Pi3k, phosphoinositide 3-kinase; Pka, protein kinase A; Pkc, protein kinase C; PMA, phorbol 12-myristate 13-acetate; ROS, reactive oxygen species; *sod2*, superoxide dismutase 2; tBHQ, *tert*-butylhydroquinone.

* Corresponding author at: Department of Biology and Ecology, Faculty of Sciences, University of Novi Sad, 2 Dositej Obradovic Square, 21000 Novi Sad, Serbia. Fax: +381 21 450 620.

E-mail address: nebojsa.andric@dbe.uns.ac.rs (N. Andric).

http://dx.doi.org/10.1016/j.toxlet.2015.10.013

Accepted 15 October 2015
Available online 19 October 2015

Keywords:
Zebrafish embryos
Erk
Akt
gstp1–2
Oxidative stress

embryogenesis is unknown. Using zebrafish embryo model, we provide novel evidence that Erk signaling acts as a specific suppressor of *gstp1–2* mRNA during early embryogenesis. Addition of Erk inhibitor U0126 enhanced *gstp1–2* mRNA expression during transition from blastula to the segmentation stage and from pharyngula until the hatching stage. Basal Erk activity did not affect *gstp1–2* expression in *tert*-butylhydroquinone-exposed embryos. Addition of phorbol 12-myristate 13-acetate increased Erk activity leading to suppression of *gstp1–2* mRNA. Activation of cAMP/Creb pathway by forskolin prevented *gstp1–2* expression, whereas U0126 suppressed Creb phosphorylation, thus setting up Creb as a proximal transmitter of Erk inhibitory effect. Collectively, these findings suggest that Erk-Creb pathway exerts suppressive effect on *gstp1–2* mRNA in a narrow developmental window. This study also provides a novel link between Erk and *gstp1–2* expression, setting apart a possible differential regulation of *gstp1–2* in adult and embryonic cells.

1. Introduction

Glutathione-*S*-transferase (GST) superfamily consists of multiple members involved in regulation of redox balance and xenobiotic metabolism. The main role of this family is transfer of the tripeptide gamma-glutamyl-cysteinyl-glycine, also known as reduced glutathione (GSH), to a wide variety of highly genotoxic and cell-damaging molecules, either directly from the extracellular environment or from the intracellular detoxification metabolism (reviewed in (Schnekenburger et al., 2014)).

The Gst Pi class (Gst Pi) belongs to the cytosolic family of GST enzymes encoded by one (human and rat) or two (mouse, zebrafish) genes (Hayes et al., 2005). Control of the Gst Pi gene (s) expression is a complex and multifactorial process, involving numerous signaling factors and signaling cascades. The presence of an antioxidant response element in GST Pi allows for activation through the nuclear factor (erythroid-derived 2)-like 2 (Nrf2) transcription factor (Taguchi et al., 2011). Two phorbol 12-myristate 13-acetate (PMA)-response elements have been identified in rat *Gstp* gene and are considered to be essential for basal and inducible *Gstp* expression (Matsumoto et al., 1999). Moreover, cAMP response element (CRE) was found in human *GSTP*, thus enabling identification of the CRE-binding protein (CREB)-dependent mechanism of *GSTP* regulation (Lo and Ali-Osman, 2002). In addition, it has been reported that extracellular signal-regulated kinase 1/2 (ERK) and protein kinase B (PKB/AKT) control rat *Gstp* expression through NRF2, AP-1 (Chang et al., 2010; Tsai et al., 2010) or NF-κB (Lin et al., 2014) transcription pathways.

Data regarding the Gst Pi class regulation have been obtained using adult mammalian cells; however, whether multifactorial regulation of the Gst Pi mRNA exists during embryonic development is completely unknown. In zebrafish, two Pi class gst genes designated *gstp1* and *gstp2* arose as a consequence of a gene duplication event (Suzuki et al., 2005). Using quantitative real-time PCR (qRT-PCR) primers that could not discriminate between the members of the Pi class, our group has previously demonstrated that expression pattern of *gstp1–2* (*gstp1* and *gstp2*) in zebrafish embryos is characterized by highly dynamic and oscillating changes due to rapid organism development. Maternal *gstp1–2* mRNA was detected at the beginning of embryogenesis, after which, the embryo's *gstp1–2* mRNA was kept at a low level until the pharyngula stage (24 hours post fertilization; hpf), then rising and reaching its maximum at the larval stage (96 hpf), followed by a sudden drop at 120 hpf (Glisic et al., 2014). Similarly to adult cells, Nrf2a has been identified as the main regulator of the *gstp1–2* expression in zebrafish embryos (Suzuki et al., 2005); however, complex fluctuations in the *gstp1–2* mRNA expression during embryogenesis and its responsiveness to a number of non-Nrf2a activating compounds (Garner and Di Giulio, 2012) implicate a diverse and multifactorial regulation of these *gstp* isoforms.

Elucidating the mechanism underlying regulation of the Gst Pi expression and the potential role of other signaling pathways is important from the aspect of redox regulation and the embryos' response to oxidative stress. Normal oscillations in redox status and GSH concentration are required for embryonic development (Timme-Laragy et al., 2013). In addition, embryonic exposure to many chemicals can generate reactive oxygen species (ROS) and cause perturbations in cellular redox status (Hansen, 2006). It is documented that the zebrafish embryos' Gst Pi class plays an important role in detoxification and metabolism of many environmental chemicals including polycyclic aromatic hydrocarbons (PAHs) and pesticides. Increase in the Gst Pi class activity or expression in zebrafish embryos has been found after exposure to PAHs (Timme-Laragy et al., 2009; Van Tiem and Di Giulio, 2011), atrazine (Wiegand et al., 2001, 2000) and polybrominated diphenyl ethers (Usenko et al., 2015) or during oxidative stress (Kobayashi et al., 2009). Moreover, it has been shown that these enzymes prevent PAH-induced toxicity in developing embryos (Garner and Di Giulio, 2012). Therefore, revealing complex signaling processes involved in regulation of the Gst Pi expression in zebrafish embryos will provide a better insight in the mechanism responsible for maintaining the redox status and protection from oxidative stress.

The goal of the present work was to understand the involvement of Mek/Erk and Pi3k/Akt signaling pathways in regulation of the *gstp1–2* expression in zebrafish embryos. We applied specific pharmacological inhibitors to identify changes in embryos obscured by early lethality in knockout or transgenic animals. In addition, this approach is attractive as small molecules/drugs can be applied and withdrawn at will, providing an alternative for expensive and time-consuming transgenic experiments. The use of signaling pathway modifying chemicals is particularly feasible in classic genetic model organisms such as *Drosophila melanogaster* and zebrafish, due to their relative low cost and the availability of a large number of fast developing embryos, which allows for testing of various concentrations and application time points (Hawkins et al., 2008). Using this approach, we revealed that Mek/Erk/Creb signaling is specifically involved in a highly dynamic regulation of the *gstp1–2* expression during zebrafish embryonic development.

2. Material and methods

2.1. Chemicals

Tert-butylhydroquinone (tBHQ), phorbol 12-myristate 13-acetate (PMA), forskolin, Erk inhibitors (U0126 and PD98059), and Akt inhibitor (wortmannin) were purchased from Sigma (Steinheim, Germany). TRIzol was obtained from Life Technologies (Carlsbad, CA, USA). All other reagents were of analytical grade.

2.2. Zebrafish care and collection of embryos

Embryos obtained from the wild type (AB strain) *Danio rerio* (eng. zebrafish) were used as a model organism in this study. Adult fish