Toxicology Letters 295 (2018) 144–152

Contents lists available at ScienceDirect

Toxicology Letters

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

Translational regulation is a key determinant of the cellular response to benzo[*a*]pyrene

Evelyn Smit, Florian Caiment, Jolanda Piepers, Jos C.S. Kleinjans, Twan van den Beucken*

Department of Toxicogenomics, GROW School for Oncology and Developmental Biology, Maastricht University, Maastricht, 6200MD, The Netherlands

ARTICLE INFO

Keywords:
mRNA translation
Benzo[*a*]pyrene
RNAseq
polysome fractionation

ABSTRACT

Translational control is a cellular response mechanism which initiates adaptation during various stress situations. Here, we investigated the role of translational control after benzo[*a*]pyrene (BaP) exposure in primary mouse hepatocytes. Translated mRNAs were separated and captured based on the number of associated ribosomes using sucrose gradients and subjected to RNA sequencing (RNAseq) to investigate translational changes. Furthermore, unseparated RNA (total RNA) was used for RNAseq to determine the transcriptional alterations. We showed that, after 24 h of exposure to 10 μM BaP, the number of genes altered by changes in mRNA translation was substantially higher compared with the number of genes altered by transcription. Although part of the BaP regulated genes were regulated by both transcription and translation, we identified genes that were uniquely regulated by mRNA translation. These mRNA transcripts encode proteins that are involved in biological processes that are not affected by transcriptional regulation. Al together this work identified a new layer of gene expression regulation that might contribute to BaP-induced carcinogenesis.

1. Introduction

On a daily basis, we are exposed to a variety of polycyclic aromatic hydrocarbons (PAHs) that are generated upon incomplete combustion of organic materials (IARC, 2012). Benzo[*a*]pyrene (BaP) is the most common PAH and is present in tobacco smoke, coal tar-based pharmaceutical products, ambient water, soils, and food (IARC, 2012; Butler et al., 1993; Hattemer-Frey and Travis, 1991). The primary exposure routes are via inhalation and food intake (Butler et al., 1993; Hattemer-Frey and Travis, 1991). After uptake, BaP is distributed to several organs including the liver which contains the highest levels of enzymes required for bioactivation. Importantly, in humans chronic exposure to BaP is associated with the development of various cancers such as lung, breast and liver cancer (IARC, 2012).

The cellular response to xenobiotics is partly regulated by changes in gene expression. Controlling mRNA translation (translational control) has been shown to be an important mechanism for modulating protein expression, especially under stressful conditions that require rapid responses including hypoxia, oncogene activation, UV and ionizing radiation (IR) (Koritzinsky et al., 2006; Powley et al., 2009; Wu et al., 2002; van den Beucken et al., 2011; Lu et al., 2006; Wahba et al., 2016; Rajasekhar et al., 2003). For example, UV irradiation causes a fast and profound decrease in *de novo* protein synthesis rates, which is time- and dose-dependent (Powley et al., 2009; Wu et al., 2002). Paradoxically, selective translation of mRNA transcripts encoding DNA damage repair proteins is increased upon UV irradiation *in vitro*, thereby promoting cellular adaptation (Powley et al., 2009). Similarly, IR promotes increased translation of mRNAs encoding for DNA repair proteins (Wahba et al., 2016). Translational control has been shown to affect 10 times more genes than transcriptional regulation after IR (Lu et al., 2006). Hypoxia affects gene expression by stimulating translation of genes involved in pH regulation, metabolism and angiogenesis, which is essential for cell survival under this stressful condition (Koritzinsky et al., 2006).

The primary mechanism of mRNA translation in eukaryotic cells is cap-dependent translation. Cap-dependent translation occurs after assembly of the eukaryotic initiation factor 4 F (eIF4 F) complex at the 5′end of a mRNA transcript. The eIF4 F complex consists of the 7-methyl-guanosine (m7G) cap binding protein eIF4E, the helicase eIF4A and scaffold protein eIF4G. Assembly of eIF4 F attracts the 43S pre-initiation complex (PIC). This complex consists out of the small 40S ribosomal subunit, the initiation factor eIF3, eIF2/GTP and initiator transfer RNA (tRNA) loaded with methionine (Merrick, 2004). Recognition of the AUG start codon within the mRNA transcript promotes the formation of the complete 80S ribosome and start of the elongation phase of translation.

Selective mRNA translation that is required for cellular adaptation to stress can be mediated via different molecular mechanisms (reviewed

* Corresponding author.
E-mail address: t.vandenbeucken@maastrichtuniversity.nl (T. van den Beucken).

https://doi.org/10.1016/j.toxlet.2018.06.009
Received 9 February 2018; Received in revised form 30 May 2018; Accepted 11 June 2018
Available online 12 June 2018
0378-4274/

in (Sonenberg and Hinnebusch, 2009)). The best described mechanisms include cap-independent translation via internal ribosome entry sites (IRES) and cap-dependent translation regulated via upstream open reading frames (uORF). Preferential translation of the pro-apoptotic protein Apaf-1 after UV exposure is dependent on an IRES in the 5′ untranslated region (5′UTR) of the Apaf-1 mRNA transcript (Ungureanu et al., 2006). In addition, under normal conditions the expression of the transcription factor ATF4 is prevented by two small uORFs present in the 5′UTR of ATF4 mRNA. Stress conditions that trigger eIF2α phosphorylation, which reduce the efficiency of translation initiation, promote bypass of the inhibitory uORFs, and ensure translation of the ATF4 encoding open reading frame (Vattem and Wek, 2004). The exposure to arsenite has been associated with ATF4 upregulation which subsequently upregulate purinic endonuclease 1 (Ape1) which is essential for the repair of the DNA damage (Fung et al., 2007).

Recognition of the m7G-cap structure by eIF4E is a highly regulated rate-limiting step in translation initiation. The availability of eIF4E is controlled by eIF4E binding proteins, 4EBP1 and 4EBP2. These limit the incorporation of eIF4E into the eIF4 F complex and inhibit mRNA translation. The interaction between eIF4E and 4EBP1/2 is dependent on the phosphorylation status of 4EBP1/2. Hyperphosphorylated 4EBP1/2 results in dissociation from eIF4E and allows assembly of the eIF4 F complex at the m7G cap of the mRNA transcript. Hypophosphorylation of 4EBP1/2 increases the affinity for eIF4E and prevents eIF4 F formation and thus initiation of mRNA translation. Phosphorylation of 4EBP1/2 is controlled by the mammalian target of rapamycin (mTOR) pathway (Zoncu et al., 2011). Under growth promoting conditions where mRNA translation is required, an active mTOR kinase stimulates 4E-BP1/2 hyperphosphorylation, while stress conditions inactivate mTOR. Formation of the eIF2/GTP/Met-tRNA ternary complex represents a second key control point for initiation of mRNA translation. The exchange of eIF2/GDP for GTP is catalyzed by eIF2B and can only occur when the eIF2α subunit is not phosphorylated at serine residue 51. Phosphorylation of eIF2α prevents the GDP/ GTP exchange and impairs general mRNA translation while allowing specific mRNA translation of genes like Activating Transcription Factor 4 (Atf4) and DNA Damage Inducible Transcript 3 (Ddit3 aka Chop) (Vattem and Wek, 2004; Young et al., 2016; Palam et al., 2011). The upstream regulators of eIF2α phosphorylation are the double-stranded RNA-activated protein kinas(eIF2 AK2 aka Pkr), PRK-like ER kinase (eIF2 AK3 aka Perk), general control non-depressible-2 (eIF2 AK4 aka Gcn2), and the heme-regulated inhibitor (eIF2 AK1 aka Hri or Hrc) (Donnelly et al., 2013). Each kinase is triggered by different kind of stressors, viral infections activate PRK, accumulation of misfolded and unfolded proteins activate Perk and, amino starvation activates Gcn2 (Donnelly et al., 2013). While translational control is recognized as an important mechanism for various kinds of cellular stresses, the importance of translational control in response to xenobiotics is poorly investigated. Therefore, we hypothesized that BaP exposure results in altered mRNA translation of specific genes. The identification of differentially translated mRNAs after the exposure to BaP may provide new insights into the carcinogenic properties of BaP. In this study, we investigated transcriptional and translational changes after BaP exposure using primary mouse hepatocytes (PMH). Well-translated polysomal mRNAs were isolated using sucrose gradients from PMH after exposure to 10 μM BaP for 1 and 24 h. Both polysomal mRNA as well as total mRNA were subjected to RNAseq analysis in order to identify differentially expressed genes. Our RNA-seq analysis showed that transcription and translation are barely affected after 1 h exposure to 10 μM BaP. However, BaP exposure for 24 h altered gene expression on the level of transcription (190 genes) and translation (623 genes). Assessing gene expression on the transcriptional and translational level identified distinct gene signatures, each representing unique cellular processes activated by BaP exposure. Many more genes associated with BaP exposure are regulated translationally than transcriptionally, thus supporting an important role for regulating mRNA translation in the response to BaP exposure.

2. Methods

2.1. Isolation and cell culture of primary mouse hepatocytes

Primary hepatocytes were isolated using a two-step collagenase perfusion. via the abdominal inferior vena cava the liver was rinsed with Hanks' calcium- and magnesium free buffer for 3 min. Subsequently, the liver was perfused with collagenase buffer (0.24 U/mL collagenase and 2.5 μM $CaCl_2$ in Hanks buffer) for 7–10 min. The buffers for the perfusion were kept at 39 °C and a perfusion rate of 7 mL/min was used. After the perfusion the liver was extracted, Glisson's capsule was removed to release the cells and the cell suspension was filter on a nylon filter. After flushing the filter with attachment medium (DMEM supplemented with 0.5 U/mL insulin, 3.5 μg/mL glucagon, 10 mg/mL hydrocortisone, 2% penicillin and streptomycin, and 5% FCS) cells were collected in 50 mL tubes and centrifuged for 3 min at 50 g and 4 °C. Subsequently, the cells were washed twice with attachment medium and cells were plated onto a collagen-coated plate. The collagen layer consisted of 1 mg/mL Collagen I rat tail (BD Biosciences, Breda, the Netherlands), 1x DMEM, and 5.8 mM NaOH. After 4 h of incubation at 37 °C and 5% CO_2 the second collagen layer was added to the culture and cells were maintained in culture medium (attachment medium without FCS). Two days after the perfusion cells were exposed to 10 μM BaP for 1 and 24 h or 0.5% DMSO for 24 h as control. Animal usage for this study was approved by the Dutch Central Commission for Animal testing (CCD).

2.2. Polysome fractionation

After BaP exposure cells were treated with 0.1 mg/mL cycloheximide (CHX) for 3 min at 37 °C, washed twice with ice cold PBS with 0.1 mg/mL CHX and harvested in PBS/CHX by scrapping. Cells were centrifuged at 300 g for 5 min at 4 °C, the cell pellet was lysed with lysis buffer (15 mM Tris (pH 7.4), 15 mM $MgCl_2$, 0.3 M NaCl, 1% Triton-X, 0.1 mg/mL CHX and 100U/mL RNase inhibitor) and incubated on ice with occasional vortexing for 10 min. Cell debris was removed by centrifugation (2000 g for 10 min at 4 °C), 10% of the lysate was stored at −20 °C in 2 volumes of 8 M guanidinium hydrochloride (G-HCL) and the remaining was transferred onto a 10 mL sucrose gradient (20–50% sucrose in 15 mM MgCl2, 15 mM Tris (pH 7.4), 0.3 M NaCl). After 2 h of centrifugation at 39,000 rpm in a Sw41-Ti rotor at 4 °C, the absorbance at 254 nm of the gradient was measured as function of gradient depth using a BioRad Laboratories UV monitor. After detection, fractions from the sucrose gradient were collected in 2 volumes of 8 M G-HCL and stored at −20 °C untill RNA isolation.

2.3. RNA isolation total RNA and from polysome fractions

After pooling the polysome fraction 40 μL of 1:1250 of the spike-in from the GeneChip® Eukaryotic Poly-A RNA Control Kit (Affymetrix) was added to the pooled fractions. RNA from the pooled fraction and the total RNA was isolated with the RNase mini kit (Qiagen) according the manufacturer's protocol starting with adding 70% ethanol after cell lysis.

2.4. Library preparation and sequencing of RNA

RNA sequence libraries were made by using the mRNA-Seq Library Prep Kit V2 (Lexogen) according to the manufacturer's protocol using 500 ng of RNA as input and 21 cycli to amplify the RNA seq library. 5 nM of cDNA from each sample was pooled and sequenced using an Illumina HiSeq 2000 in 100 bp paired-end reads. Raw RNAseq data are available on array express E-MTAB-6465. The quality of the sequence data was analyzed by means of FastQC.V0.011.5.